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Final Report:

Evaluation of the Occupational Risks from Jet Fuel (Toxicity Screening Battery)

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14. ABSTRACT
Studies are needed to identify the potential toxicity of alternative jet fuels and to develop occupational exposure limits (OELs). A full toxicity program is costly and time consuming. Currently, there are too many novel alternative and bio-based jet fuels being rapidly developed to conduct full toxicity programs for each new fuel. The goal of this study was to design three *in vitro* approaches suitable for use as screening tests for the fuel evaluation process. A cytokine screen was used to examine blood from rats exposed to an alternative jet fuel. Although cytokines were detected, the data were inconsistent and highly variable. A more refined array of cytokines is needed. Development of an *in vitro* screen for lung cells could replace or reduce the number of inhalation studies required to screen bio-based jet fuels. A previously developed co-culture was successfully adapted for used with alternative jet fuels. This *in vitro* method of exposing human lung and immune cells, in conjunction with mitochondrial viability and cytokine measurements, showed promise as a means of rapidly assessing fuel toxicity and effects on the lung. Two human cell models also were evaluated for their potential to properly assess dermal irritation caused by jet fuel. The first model was a commercially available three-dimensional human skin equivalent model which was not predictive for jet fuels. The second model was a human epidermal keratinocyte cell line isolated from adult human skin, which showed potential for identifying dermal irritation.

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Introduction

The initial evaluation of a new fuel involves identifying components of the fuel, performing literature searches on the fuel and its components for any known toxicity data, and identifying/verifying the research that is necessary to complete the development of exposure standards. For bio-based jet fuels, there is no toxicity data available as these are new fuels. Although efforts are underway to analytically compare these new fuels with JP-8, such an approach is difficult due to the high number of hydrocarbon components, many of which do not have analytical standards with which to either identify them or quantitate the amount present. Therefore, toxicity screens are needed to identify any potential toxicity of bio-based fuels and to develop an occupational exposure limit (OEL) based on their comparison to the fully characterized jet fuels, Jet Propulsion (JP)-8 and S-8 (Fischer-Tropsch jet fuel). S-8 is the only alternative fuel that presently has sufficient toxicity data to develop an OEL (Mattie et al., 2010). Currently, there are too many novel alternative and bio-based jet fuels being rapidly developed to conduct the Fischer-Tropsch (F-T) program on each new fuel. In an effort to streamline the toxicity testing process, there are studies in progress to identify the necessary toxicity tests for screening new fuels and incorporate reliable new methods aimed at rapidly and economically evaluating new fuels.

The goal of this project was to design and begin to integrate *in vitro* approaches suitable for screening tests leading into the fuel evaluation process. The overlying hypothesis is that *in vitro* screens for respiratory and dermal effects, in addition to cytokine profiling in the blood, can aid in the prediction of exposure limits for alternative fuels when the data are compared to existing baseline toxicity data. To evaluate this hypothesis, additional studies were needed to develop and assess the *in vitro* methods

for collecting suitable and reliable data that can be used for *in vitro – in vivo* comparisons. Toward this end, three main objectives/phases were proposed:

1.) Cytokine screen: Inhalation studies with S-8 identified the nasal cavities and lungs as primary target organs. Exposures planned or in progress will evaluate the lungs for changes in cytokines so that the appropriate cytokines can be incorporated into the *in vitro* screen for lung cell toxicity. The blood from the rats in this study could also be screened for cytokines that may serve as biomarkers or a molecular profile in the blood of animals in future studies or for testing operational personnel for exposure to JP-8, F-T or bio-based jet fuel.

2.) Development of an *in vitro* screen for lung cells to replace or reduce the number of inhalation studies required to screen bio-based jet fuels: A previously developed co-culture with the potential to screen toxic chemicals and materials will be used to test jet fuel toxicity *in vitro*.

3.) Development of an *in vitro* screen for dermal irritation to replace rabbit studies: Currently, dermal irritation studies have been or are being conducted for jet fuels using rabbits. There are *in vitro* screens available for human dermal irritation; however, the data in the literature is questionable as to their relevance for potential irritation of jet fuels in humans. Specific testing of jet fuels using one or more of these human dermal models is needed to determine if *in vitro* screening can be used in the future for predicting dermal irritation of bio-based jet fuels.

Phase I. Cytokine Screen

The nasal cavities and respiratory system (i.e., lungs) have been identified as the primary target organ for jet fuel exposure. Such exposure has resulted in localized inflammation, a process often mediated by cytokines, small cell-signaling proteins secreted by cells. Production and secretion of cytokines by cells serve as a means of intercellular communication, thereby directing the immune system in both pro- and anti-inflammatory events. The goal of this phase was to evaluate changes in cytokine levels due to jet fuel exposure via inhalation. Ideally, a change in cytokine production/release (increase or decrease) has the potential to develop a “fingerprint” of exposure that can be used to assess toxicity associated with jet fuel exposure. Such a tool would prove useful in animal models and in determining occupational exposures in military personnel (via blood samples). Additionally, a cytokine profile developed from an animal model can be used to validate *in vitro* or cell-based models should the cytokines secreted by cells in laboratory models reflect the same changes following jet fuel exposure. Should cell-based models be suitable, they would greatly increase the rapidity and decrease the cost of evaluating the potential toxicity of newly developed jet fuels.

For this study, a series of cytokines involved in inflammation were evaluated in the blood of rats previously exposed to the alternative jet fuel R-8 (Hydro-processed Renewable Jet (HRJ) from fats and oils (AFO)). Due to availability, the following pro-inflammatory cytokines were chosen as part of a multi-plex approach: Interferon-γ (IFN-γ), Interleukin (IL)-1β, IL-4, IL-5, IL-13, KC/GRO (IL-8), and Tumor Necrosis Factor-α (TNF-α).

Methods

Samples: Serum samples from fuel-exposed rats were provided by the Fuels Branch, AFRL/RZPF at Wright-Patterson AFB, OH. Male and female rats (5 per group) were exposed via inhalation (by The Hamner Institutes for Health Sciences, Triangle Park, NC) to low (200 mg/m^3), medium (700 mg/m^3) and high (2000 mg/m^3) concentrations of the alternative jet fuel, R-8. Control rats were unexposed. Blood was drawn post-exposure and the serum samples were stored at -80°C. The following samples were provided (note: due to sample number limitations, serum samples for rats exposed to the low concentration of R-8 were not analyzed):

Cytokine analysis: Serum samples were thawed and analyzed for cytokine levels using a multiplex approach with the Meso Scale Discovery Rat Demonstration 7-plex Ultra-Sensitive Kit (Lot number K0032519 MSD, Gaithersburg, MD). This plate analyzed the following cytokines: IL-1β, KC/GRO (IL-8), IL-4, IL-5, IFN-γ, TNF-α, and IL-13. Each sample was analyzed in duplicate according to manufacturer's instructions. Briefly, an 8-point calibration curve of 40000, 10000, 2500, 625, 156, 39, 9.8, and 0 pg/ml was prepared using the 1 mg/ml calibrator stock. 25 µl of Diluent 6 was added to each well of the 96-well plate and the plate was sealed and incubated for 30 min with vigorous shaking at room temperature. 25 µl of calibrator or sample was added per well and the plate was sealed and incubated at room temperature with vigorous shaking for 2 h at room temperature. The plate was washed 3 times with phosphate buffered saline with 0.05% Tween-20 (PBS-T) and 25 µl of the 1X Detection Antibody Solution was added to each well. The plate was sealed and incubated with vigorous shaking for an additional 2 h at room temperature. The plate was washed 3 additional times with PBS-T. 150 µl of

prepared Read Buffer T was added per well and the plate was analyzed using the SECTOR Imager (MSD, Gaithersburg, MD).

Statistical analysis: Means and standard deviations were determined for each cytokine from exposure groups for males and females, separately. Two-tailed *t*-tests were performed to compare cytokine levels in serum from fuel-exposed rats to the unexposed controls.

Results and Discussion

All cytokines were detectable in each of the serum samples from control, medium and high concentration R-8 exposed rats (Figures 1 through 7; raw data provided in Appendix A). Significant changes were seen with IFN-γ, TNF-α, IL-4, IL-5 and IL-1β, however in most cases the changes were not consistent across gender. For example, male rats displayed a significant, albeit small, increase of IFN-γ in the blood of fuel exposed animals while female rats exposed to the high concentration of fuel exhibited a decrease (Figure 1). A similar trend was also observed with TNF-α (Figure 4). Interpretation is also complicated by large standard deviations between samples within the same group. Pathological analysis (separate study) indicated a level of inflammation in a number of the control animals, which may have contributed to elevated cytokine levels in the controls, thereby decreasing the difference between control and exposed animals.

Overall, the changes in cytokines show promise that a cytokine profile may offer a means of assessing toxicity in blood/serum samples, with the potential to transfer the exposure to non-animal, cell-based models. Evaluation of other cytokines and at

different times during or post-exposure (cytokines fluctuate to initiate or reduce inflammation) and exposure to different fuels (the potential for a greater response) would aid in advancing this approach. Cytokines of particular interest include the pro-inflammatory cytokines IL-6 and GM-CSF, the latter of which has shown potential in cell-based models, and IL-10 that is involved in reducing inflammation.

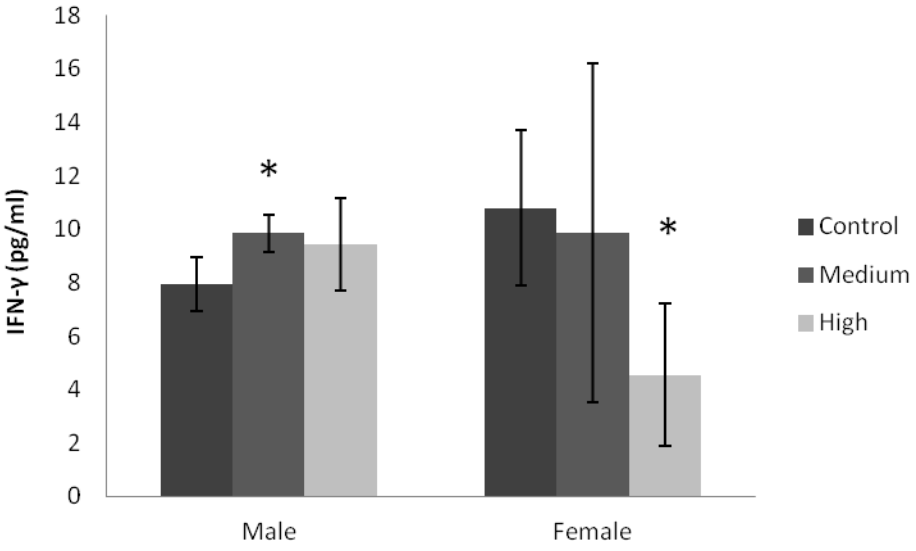


Figure 1. Concentration of IFN-γ in male and female rat serum samples exposed to the jet fuel R-8 by inhalation. *, p-value <0.05.

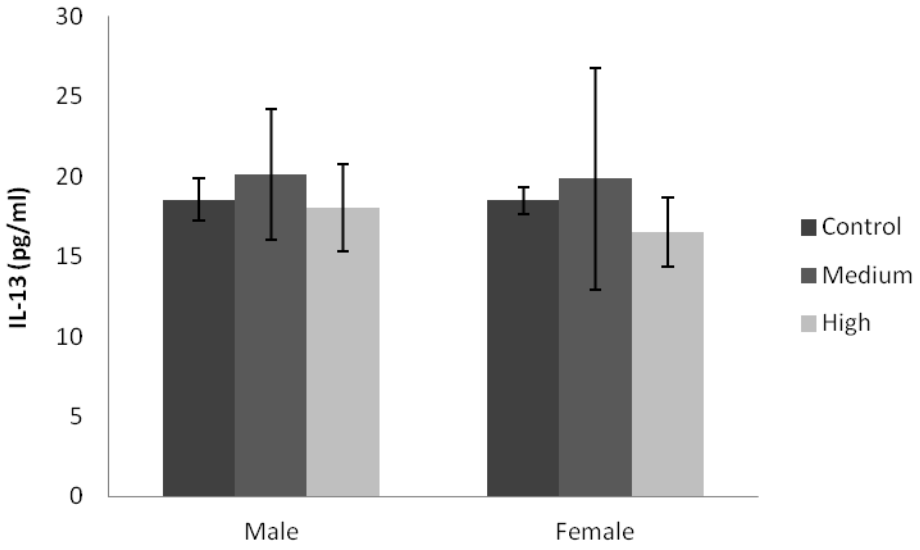


Figure 2. Concentration of IL-13 in male and female rat serum samples exposed to the jet fuel R-8 by inhalation

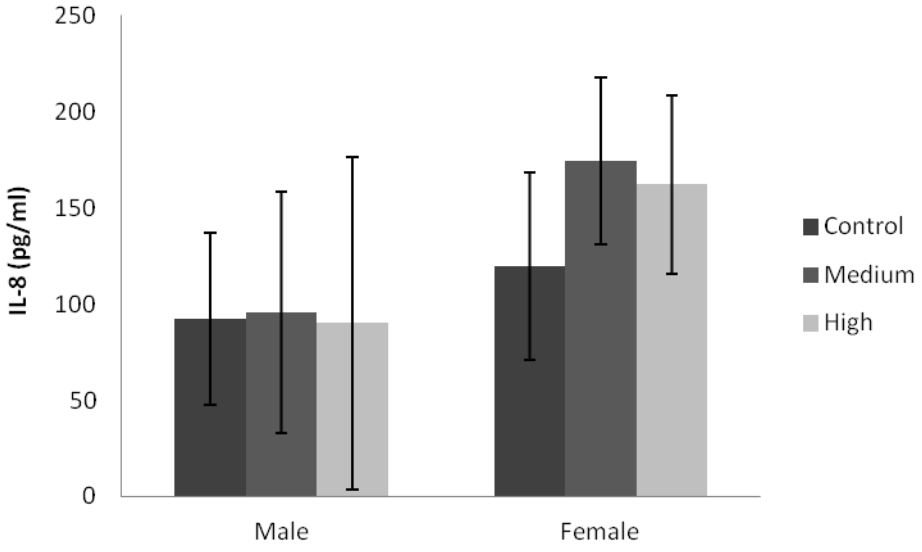


Figure 3. Concentration of IL-8 in male and female rat serum samples exposed to the jet fuel R-8 by inhalation.

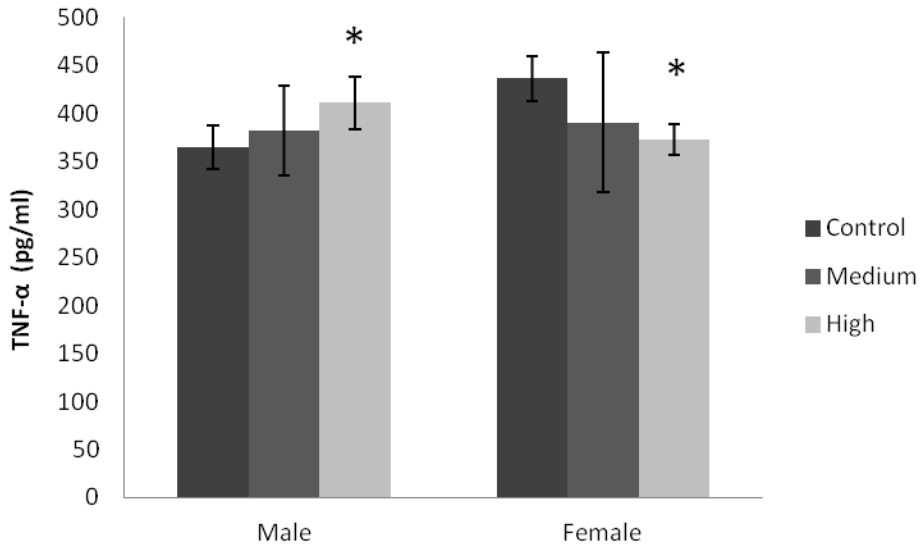


Figure 4. Concentration of TNF-α in male and female rat serum samples exposed to the jet fuel R-8 by inhalation. *, p-value <0.05.

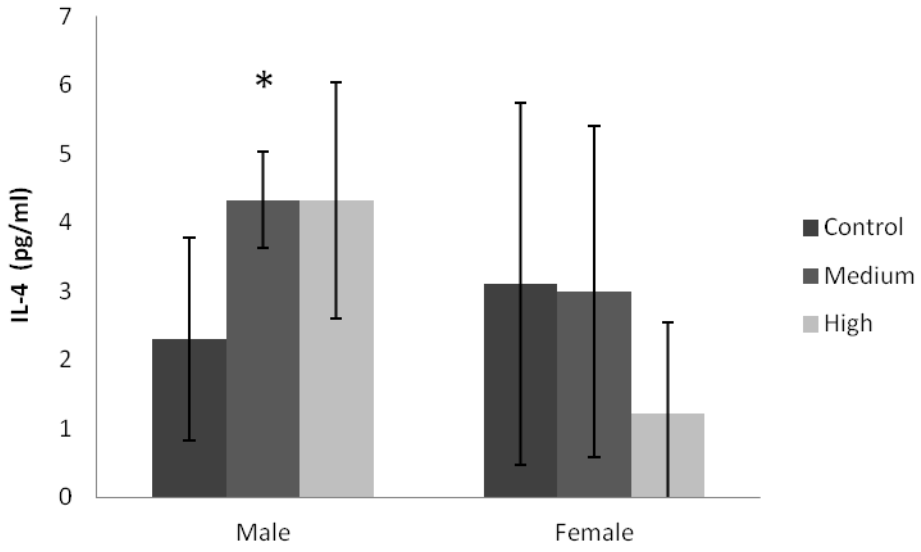


Figure 5. Concentration of IL-4 in male and female rat serum samples exposed to the jet fuel R-8 by inhalation. *, p-value <0.05.

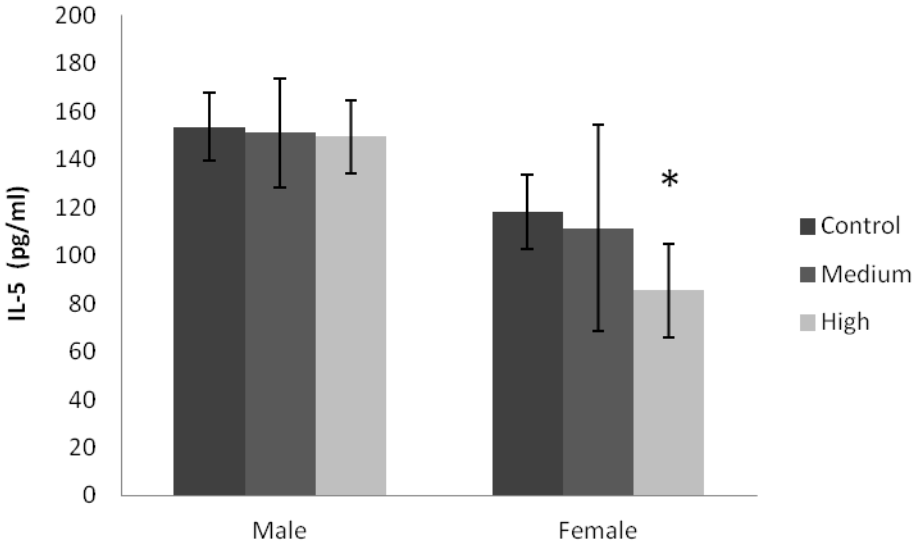


Figure 6. Concentration of IL-5 in male and female rat serum samples exposed to the jet fuel R-8 by inhalation. *, p-value <0.05.

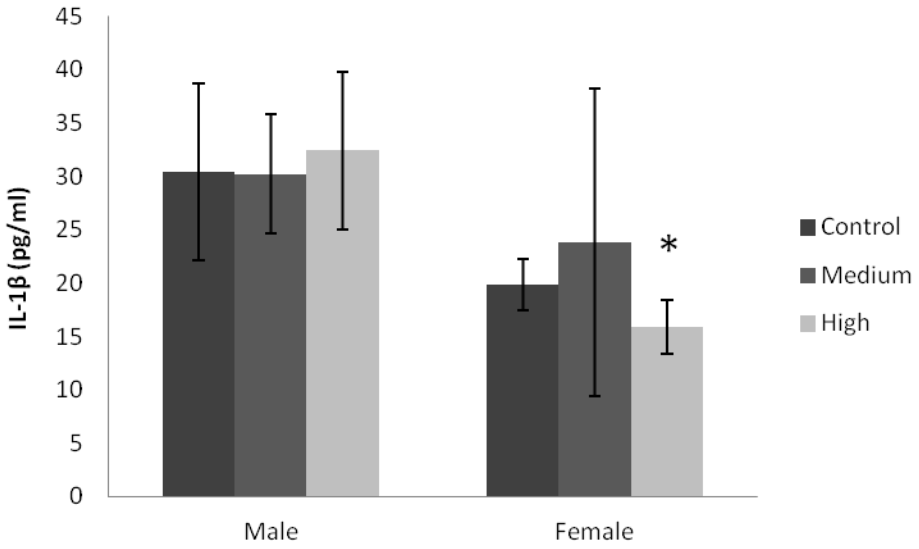


Figure 7. Concentration of IL-1β in male and female rat serum samples exposed to the jet fuel R-8 by inhalation. *, p-value <0.05.

Phase II. In Vitro Lung Screen

The nasal cavities and respiratory system (i.e., lungs) have been identified as the primary target organ for jet fuel exposure. Typically, inhalation studies utilizing animal models have been employed to evaluate the toxicity of jet fuels. However, these studies are costly and time consuming. While *in vitro* models may not yet be advanced enough to replace animal inhalation studies, these models can be employed as a screening method to rapidly and cost effectively acquire initial toxicity data on new fuels using relevant systems, such as those comprised of human cells.

The goal of this phase was to develop an *in vitro* screening method consisting of human cells (lung and immune cells) that, upon/following exposure to jet fuels, yield data that can be used to evaluate the potential toxicity of the fuels and determine whether the fuel should be further evaluated for toxicity. This study exposed co-cultures of human lung cells and macrophages to jet fuel and evaluated the effects on the cultures by both mitochondrial viability and changes in cytokine levels produced and released into the media. Similar to Phase I, a change in cytokine production/release (increase or decrease) has the potential to develop a “fingerprint” of exposure that can be used to assess toxicity associated with jet fuel exposure. Should these *in vitro* screens prove successful, they would greatly increase the rapidity and decrease the cost of evaluating the potential toxicity of newly developed jet fuels.

For this study, a series of cytokines involved in inflammation were evaluated in the growth media of the cells following exposure to jet fuel. Due to availability, the following pro-inflammatory cytokines were chosen as part of a multi-plex approach:

Granulocyte macrophage colony-stimulating factor (GM-CSF), Interleukin (IL)-1β, IL-2, IL-5, IL-6, IL-8, and Tumor Necrosis Factor-α (TNF-α).

Methods

Jet fuels: The following jet fuels were provided by the Fuels Branch, AFRL/RZPF at Wright-Patterson AFB, OH and tested in the *in vitro* lung screen. The densities shown were determined with the provided samples in order to calculate the concentration (µg/ml) of fuel added to the media for direct exposure (described below).

Jet fuel (as labeled)	Density (g/ml)
JP-8 (baseline)	0.782
S-8	0.730
R-8	0.736
Amyris	0.792
Swedish Biofuel	0.736
R-8 from Algae	0.735

Tissue culture: To evaluate the effects of jet fuel on the human lung, an *in vitro* model was employed and consisted of a co-culture of two human cell lines in order to mimic the alveoli environment (Braydich-Stolle et al., 2010; Wang et al., 2002). The lung epithelial cell line, A549 (CCL-185; American Type Culture Collection, Manassas, VA) was cultured in the presence of stimulated human monocytes, U937 (CRL-1593.2; American Type Culture Collection, Manassas, VA). The individual cell lines were cultured separately in RPMI-1640 media supplemented with 1% penicillin-streptomycin solution and 10% heat inactivated fetal bovine serum at 37°C with 5% CO_2. Prior to

seeding experimental wells, the U937 cells were differentiated into macrophages by stimulation with 100 ng/ml phorbol-12-myristate-13-acetate (PMA, Sigma, St. Louis, MS) for 48 hours. Following stimulation, A549 and U937 cells were plated in the same wells of a 24-well tissue culture plate at a ratio of 3 epithelial cells to 1 macrophage. Experiments were performed following 24 h, allowing time for the cells to adhere to the wells.

Jet fuel exposure: Co-cultures were exposed to jet fuel either directly with exposure by dilution of fuel into the media or atmospherically by allowing jet fuel to volatilize in the presence of the cells (i.e., vapor exposure) for the indicated amount of time, as described below. Following exposure, media was collected from each well and stored at -20°C until processed for cytokine analysis. Mitochondrial viability (via the MTT assay) was measured on the remaining cells as an indicator of cell viability. Briefly, following removal of the media from the cells, methylthiazolyldiphenyl-tetrazolium bromide (MTT; 5 mg/ml phosphate buffered saline) was diluted 1:10 in media and 500 µl of the solution was added to the cells and incubated at 37°C and 5% CO_2 for 4 hours. The solution was aspirated and 200 µl dimethyl sulfoxide (DMSO) was added to solubilize the cells. 800 µl 0.1M glycine-0.1M NaCl solution (pH 10) was added and the contents of the well were thoroughly mixed and 200 µl were transferred to a 96-well plate and the absorbance measured at 570 nm. Control wells were considered to be 100% viable and the experimental conditions were normalized to the controls.

Direct exposure: Jet fuel was diluted in DMSO to a stock concentration of 200 mg/ml made immediately prior to use. This stock was further diluted to 200, 20, 2, and

0.2 mg/ml in the vehicle so that final concentrations of 100, 10, 1, and 0.1 µg/ml were reached with a 1:2000 dilution into RPMI-1640 media. To apply to the cells, media was aspirated from wells containing cells and replaced with 0.5 ml of warm media containing jet fuel. Media and media with the vehicle (1:2000) served as controls and each condition was performed in quadruplicate (n=4) on the same 24-well plate. Plates were incubated for approximately 4 h at 37°C and 5% CO_2.

Vapor exposure: A549 and U937 were plated together in the 8 wells (n=8) adjacent to the 4 center wells of a 24-well tissue culture plate. 1 plate was seeded per fuel or control. The cells were allowed to adhere overnight. Glass inserts were placed in the 4 center wells of each plate and 25 µl of fuel was added to the inserts. Plates were sealed with parafilm and incubated for 18 h at 37°C and 5% CO_2 to allow the fuel to vaporize and generate a fuel atmosphere. Controls were incubated in a separate incubator. These vapor exposures were performed to achieve a “proof of concept” that a form of vapor exposure is an amenable approach for cellular systems and fuel screening efforts. While the concentration of vaporized fuel was not quantitated during these experiments and would be difficult to estimate, future efforts will focus on generating and delivering a defined concentration of jet fuel aerosol.

Cytokine analysis: Tissue culture supernatant samples were thawed and analyzed for cytokine levels using the Meso Scale Discovery Human Demonstration 7-plex Cytokine Assay for Tissue Culture (Lot number Z0043140 MSD, Gaithersburg, MD). At least three replicates were randomly chosen from each experiment and analyzed in duplicate according to manufacturer’s instructions. Briefly, an 8-point calibration curve of 10000,

2500, 625, 156, 39, 9.8, 2.4, and 0 pg/ml was prepared using the 1 µg/ml calibrator stock. 25 µl of calibrator or sample was used per well and the plate was sealed and incubated with vigorous shaking for 1.5 h at room temperature. 25 µl of the 1X Detection Antibody Solution was added to each well and the plate was sealed and incubated with vigorous shaking for an additional 1.5 h at room temperature. The plate was washed 3 times with phosphate buffered saline with 0.05% Tween-20. 150 µl of prepared Read Buffer T was added per well and the plate was analyzed using the SECTOR Imager (MSD, Gaithersburg, MD).

Statistical analysis: Means and standard deviations were determined for each condition for mitochondrial viability and cytokine levels. Two-tailed *t*-tests were performed to compare fuel exposed cells to the unexposed controls.

Results and Discussion:

Two different methods were employed to expose the co-cultures to the fuel. The first method involved diluting the fuel into the media (Figures 8 through 12). While known concentrations are delivered, the fuel is not stable in the liquid phase of the media and therefore contact or interaction between the fuel and the cells is uncertain. Furthermore, volatilization of the fuel components is also rapidly occurring and therefore certain constituents of the fuel are not likely to remain in the liquid media. To limit the effects of these events, and based on previous unpublished studies, the exposures were restricted to 4 h.

Despite a short exposure time, there were mild decreases in mitochondrial viability when the co-cultures were exposed to JP-8 (Figure 8). However, only the 10 µg/ml concentration yielded a statistically significant decrease. On the contrary, the remaining fuels tested by this method (Amryis, S-8, R-8, R-8 from Algae and Swedish Biofuel) did not induce any loss in mitochondrial viability in the co-cultures over the course of 4 h (Figures 9 through 13).

JP-8 and three additional fuels, S-8, Swedish Biofuel and R-8, were also evaluated for their ability to reduce mitochondrial viability in the co-cultures via vapor exposure where the fuel is allowed to volatilize in the confined presence of the cells for 18 h. Previous unpublished data has indicated that 18 h is the optimal time for this exposure, allowing for ample volatilization without restricting cell activity due to the confined atmosphere (i.e., O_2/CO_2 exchange). Under this exposure scenario, JP-8 results in a 30% loss in the mitochondrial viability of the co-cultures, while co-cultures exposed to the other three fuels did not display a loss in viability (Figure 14). In fact, those co-cultures exposed to R-8 experienced an unexplained increase in mitochondrial viability during that time, indicating that significant cellular activity is capable of occurring in this exposure system. Taken together, these results indicate that both of these exposure methods yield an effect on the lung and macrophage co-culture, albeit small for the direct exposure method, when exposed to JP-8. Therefore, these methods show potential for comparison of new fuels to the conventional fuel, JP-8. Additional studies would be needed to better define the vapor atmosphere (i.e., concentration and constituents), more extensively optimize and validate these methods to determine the upper and lower boundaries of exposure and maximize endpoint measurements so that

minor differences between fuels can be distinguished. This may involve secondary endpoints including lactate dehydrogenase (cell permeability) measurements or barrier function in a further developed model incorporating polarized monolayers of the lung epithelial cell (with macrophages).

Levels of a series of cytokines, GM-CSF, IL-1β, IL-2, IL-5, IL-6, IL-8, and TNF-α, were also evaluated in the media of subset of samples from direct and vapor exposed co-cultures (Tables B-1 through B-14 of Appendix B). Low levels were observed in media collected from the direct exposure method; however, this may likely be the result of the shortened duration of time in which the cells were able to secrete the cytokines (4 h vs. 18 h). One cytokine, GM-CSF, showed a significant increase with exposure to JP-8, Swedish Biofuel and R-8, but not S-8, indicating that this cytokine may reflect or correlate to jet fuel exposure (Figure 15). Method optimization could result in greater levels of cytokine production and secretion and allow for further differences to be observed between fuel exposed co-cultures. Overall, these cell-based *in vitro* methods of exposing human lung and immune cells, in conjunction with mitochondrial viability and cytokine measurements, show promise as a means of rapidly assessing fuel toxicity and effects on the lung.

Future developments will focus on refining the physiological relevance of the lung model and developing a system in order to control and quantify the jet fuel vapor/aerosol exposures. Control over the concentration for the atmospheric exposures will allow for clear determinations of no observed adverse effect levels and maximum tolerated doses for comparison between fuels.

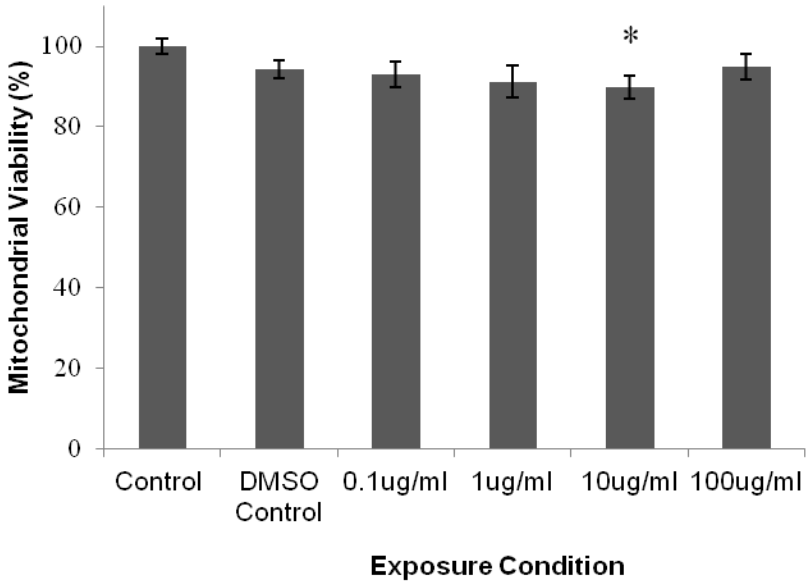


Figure 8. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to JP-8 at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. *, p-value <0.05 in comparison to the media control.

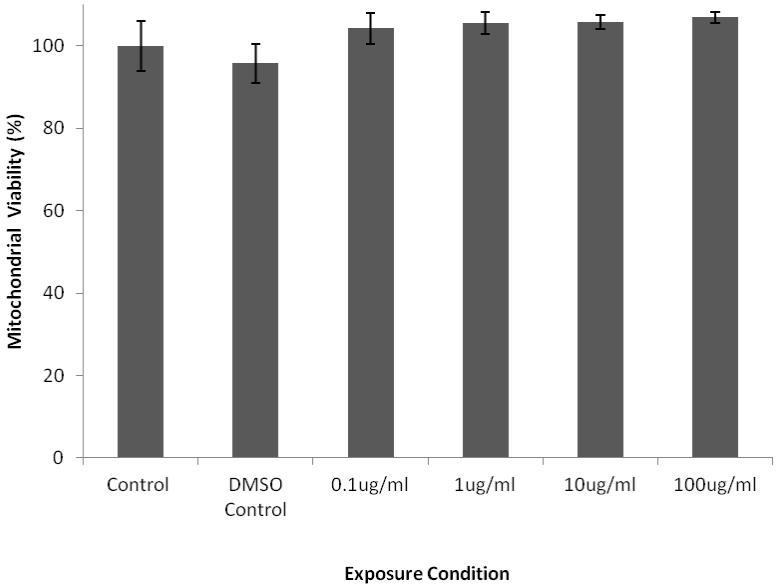


Figure 9. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to Amyris fuel at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. There were no significant differences in comparison to the media control.

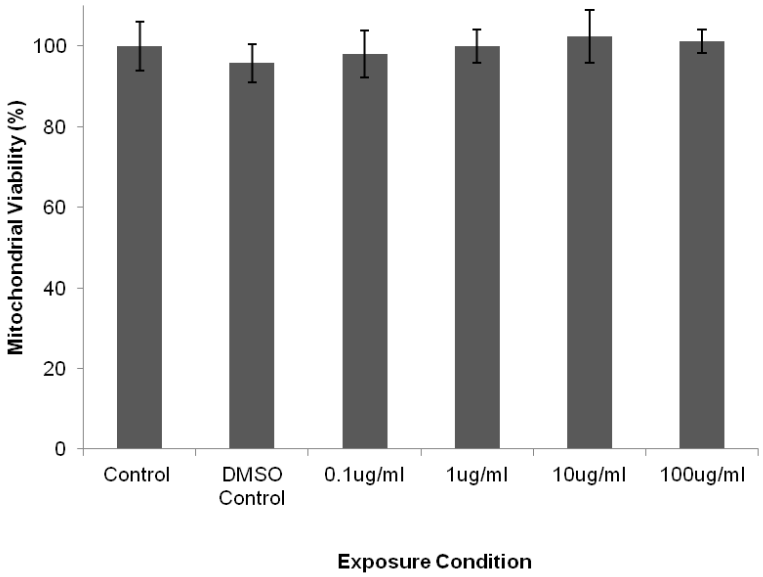


Figure 10. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to S-8 at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. There were no significant differences in comparison to the media control.

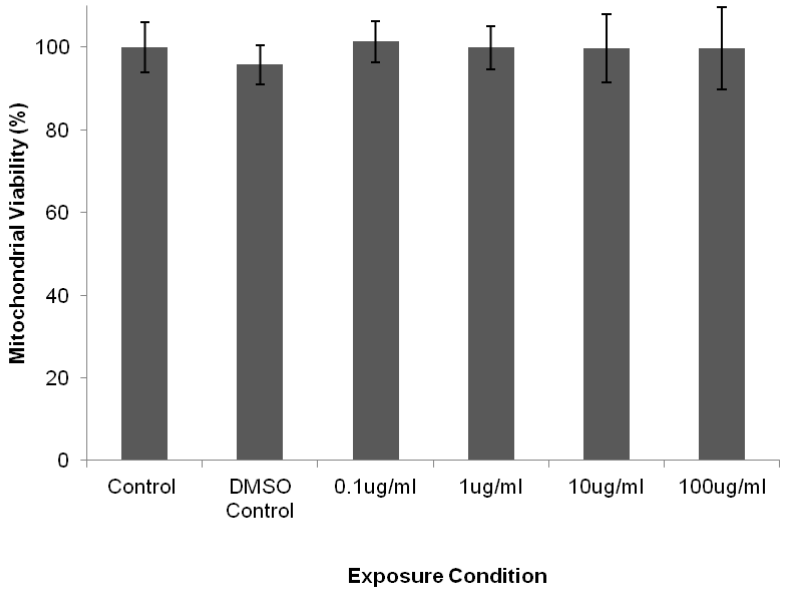


Figure 11. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to R-8 at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. There were no significant differences in comparison to the media control.

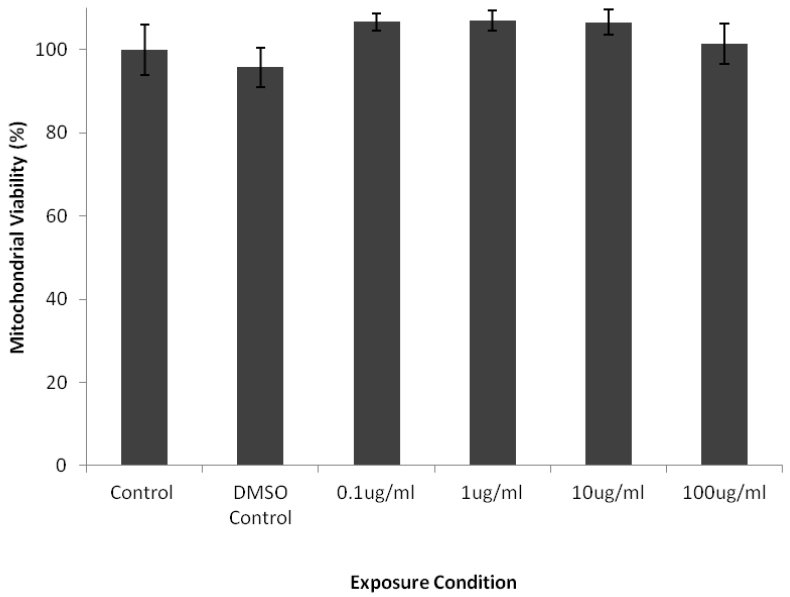


Figure 12. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to R-8 from algae at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. There were no significant differences compared to the media control.

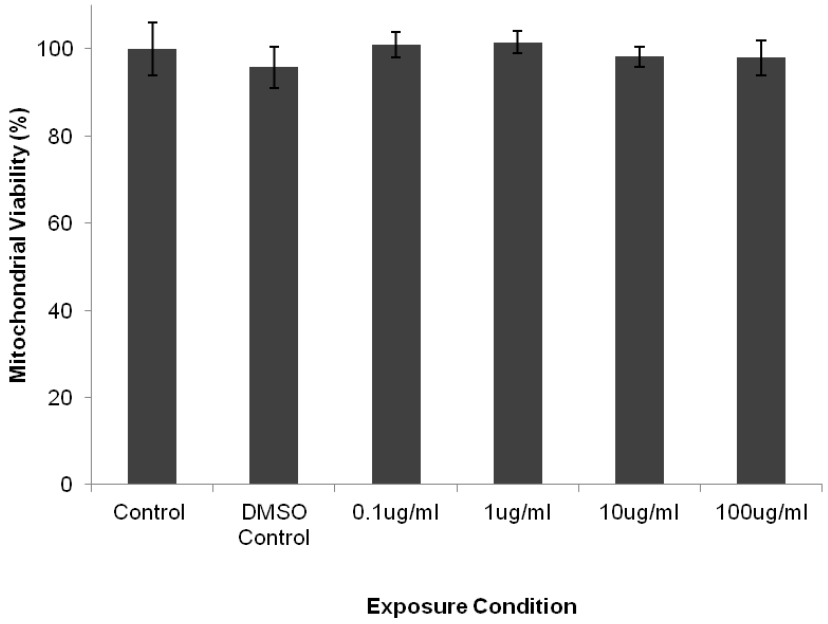


Figure 13. Mitochondrial viability of A549:U937 co-cultures following 4 h exposure to Swedish Biofuel from algae at the concentrations shown. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. There were no significant differences in comparison to the media control.

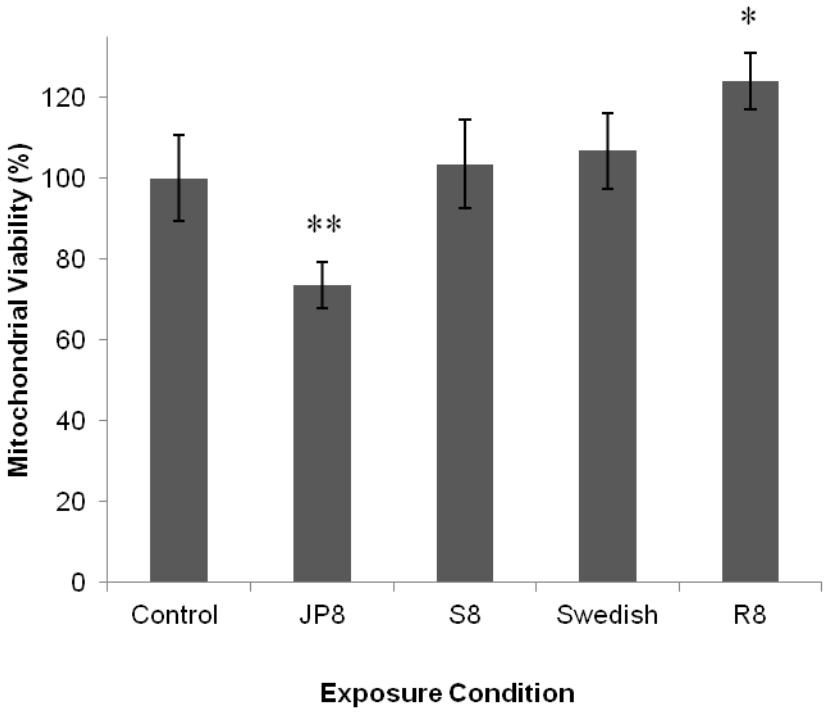
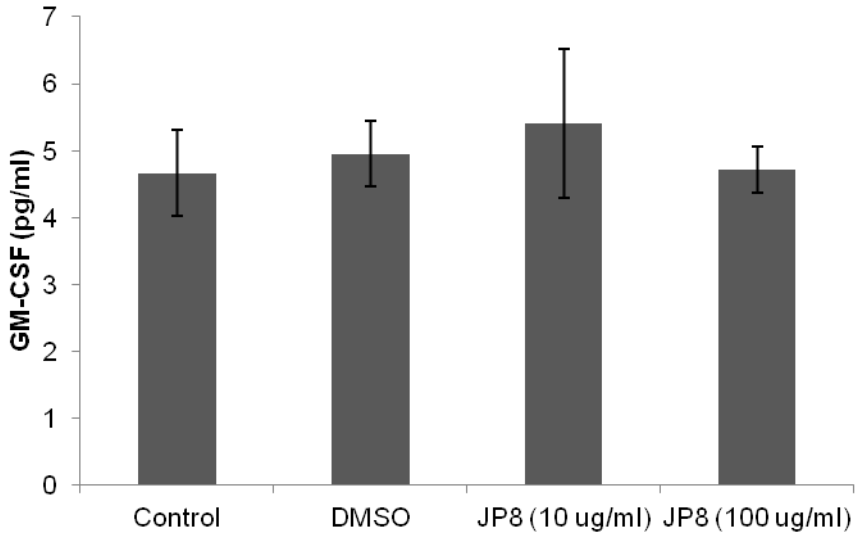


Figure 14. Mitochondrial viability of A549:U937 co-cultures following 18 h exposure to JP-8, S-8, Swedish Biofuel or R-8 by vapor exposure. Data are the means ± standard deviations (error bars) of quadruplicate samples and represent one of at least two experiments in which similar results were obtained. *, p-value <0.05; **, p-value <0.0001 in comparison to the media control.

A



B

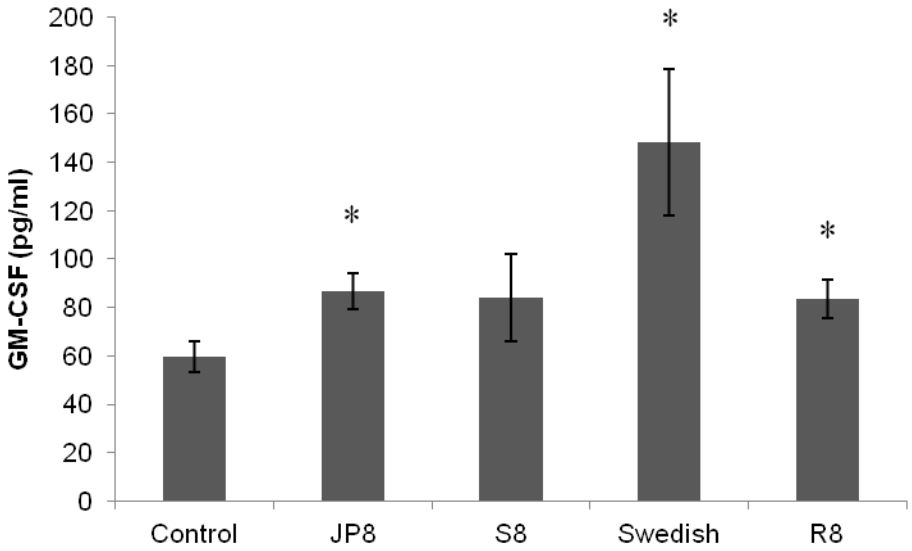


Figure 15. Levels of the cytokine GM-CSF released into media during exposure to jet fuel by the direct exposure method for 4 h at the concentrations of JP-8 shown (A) or the vapor exposure method for 18h for JP-8, S-8, Swedish Biofuel and R-8 (B). Data are the means ± standard deviations (error bars) of triplicate samples. *, p-value <0.05; in comparison to the media control.

Phase III. In Vitro Dermal Irritation Screen

Dermal irritation caused by jet fuel, and many other substances, is currently determined through the use of rabbits. While this method of evaluating skin irritation is widely accepted, there is a significant drive to utilize alternative or non-animal models when possible. In addition to reducing the number of animals being used in research, *in vitro* models offer advantages over animal models (when suitable) by significantly decreasing the expense and time associated with testing, as research animals can be costly and the experiments time consuming. Recent advances in cell-based models have resulted in the development of multiple dermal models and their incorporation is gradually being accepted by research communities. These models are highly cost effective and the procedures are relatively rapid compared to the time investment in preparation of animal studies. Furthermore, the *in vitro* models are comprised of human cells, and therefore may be more informative of the potential risk. This study aimed to evaluate *in vitro* models of dermal irritation for use with jet fuel. Toward this end, two human cell models were evaluated for their potential to properly assess dermal irritation caused by jet fuel. The first model was a commercially available 3-dimensional human skin equivalent model comprised of differentiated cells that stratify into 8-12 cell layers (basal, spinous, and granular layers) and 10-15 layers of stratum corneum, similar to the human dermis. The second model was a human epidermal keratinocyte cell line isolated from adult human skin.

Methods

Jet fuels: The jet fuels were provided by the Fuels Branch, AFRL/RZPF at Wright-Patterson AFB, OH. Jet fuel samples were JP-8 (for baseline purposes), Jet A Blend, Sasol IPK, Sasol GTL-1, Sasol GTL-2, Shell GTL, S-8, R-8, Amyris, Swedish Biofuel, R-8 from Algae, and HRJ (from camelina).

3-dimensional human skin equivalent model: The EpiDerm Skin Model (EPI-200; Mattek, Ashland, MA) is a multi-layer dermal model that is 9mm in diameter. Exposures and analysis were performed in accordance with manufacturer's instructions and based on Kandarova et al., 2009. Undiluted jet fuel (50 µl, unless otherwise indicated) was placed directly on the equilibrated tissues and incubated for 24 h, unless otherwise indicated. Controls included assay medium (30 µl), supplied Dulbecco's Phosphate-Buffered Saline (DPBS; 30 µl), 5% sodium dodecyl sulfate (SDS; 30 µl; known irritant), isopropanol (30 µl; known mild irritant), and 1% Triton-X (30 µl; known moderate-mild irritant). All conditions were tested in triplicate. Mitochondrial viability was determined by the MTT assay using the supplied reagents, per manufacturer's instructions. Media/buffer controls were set to 100% viability.

Levels of IL-1α were assessed in media collected during the medium exchange step on day 2 of the procedure and stored at -20°C until use. Concentrations of IL-1α were determined using the Platinum ELISA kit for IL-1α (eBioscience, San Diego, CA), in accordance with manufacturer's instructions.

Human epidermal keratinocyte model: Adult human epidermal keratinocyte (HEKa) cells were purchased from Invitrogen (Carlsbad, CA). Cells were thawed and maintained in EpiLife Medium supplemented with Human Keratinocyte Growth Supplement, according to the supplied product information sheet. Cells recovered from cryopreservation were seeded at a density of 2.5 x 10^3 viable cells/cm^2 in 75 cm^2 culture flasks. Upon reaching 80% confluency, cells were subcultured once (~1 week after thawing) and new flasks seeded with ~4 x 10^5 cells. At 80% confluency, cells were seeded in 96-well plates with ~5 x 10^3 cells/well within 1 week of subculturing. Seeded cells were allowed 2-3 days to adhere to the plate surface and recover prior to fuel exposure.

40 µl of each control (media, isopropanol, 1% Triton, 5% SDS) or fuel was added directly to the media (100 µl) in each of four wells (n=4) of a 96-well plate. After 10 minutes, all of the liquid in each well was aspirated out and each well was washed once with 100 µl media. 200 µl of media was added to each well, and the plates were incubated at 37°C, 5% CO_2. 4 h incubation time was found to be the optimal incubation time (vs. 0, 8 and 24 h, data not shown). Following incubation, mitochondrial viability was assessed using the MTT Cell Proliferation Assay (ATCC, Manassas, VA) in accordance with manufacturer's instructions. Briefly, 100 µl of media plus 10 µl of MTT reagent was added to each well and the plates were incubated 2-4 h. When a precipitate was observed, 100 µl of detergent was added per well and the plate was mixed gently, covered and incubated at room temperature 2-4 hours or overnight prior to reading the absorbance at 570 nm.

Statistical analysis: Means and standard deviations were determined from the replicates for each individual condition. Two tailed *t*-tests were performed to compare each condition to the media/buffer controls.

Results and Discussion

In order for an *in vitro* dermal model to be useful in predicting dermal irritation caused by exposure to new fuels, it should appropriately mimic the responses found with fuels previously tested in an *in vivo* dermal model. JP-8 is categorized as a slight to moderate irritant, with variable effects in rabbit models and a wide range of effects in humans occupationally exposed. This creates a challenge when developing or modifying an *in vitro* model, as the "appropriate" response of the model may be difficult to identify. Given this, a series of optimization steps were taken to determine proper dosage and exposure time of the 3-dimensional tissue to JP-8. Controls included the assay medium, 5% SDS (known irritant), isopropanol (known mild irritant), and 1% Triton-X (known moderate-mild irritant). JP-8 was added to the tissues at increasing volumes for an exposure time of 4 h with no reduction in mitochondrial viability observed in comparison to the media control (Figure 16). The highest volume of fuel (50 µl) was then applied to another set of tissues for 8 and 16 h, with no reduction in mitochondrial viability (Figure 17). 24 h and 40 h exposure times to 50 µl of JP-8 were also tested, with no reduction in mitochondrial viability observed (data not shown). During one trial with JP-8, the procedure was modified so that exposure occurred for 24 h followed immediately by the MTT assay instead of the recommended 24 h post-exposure

incubation. However, this modification to eliminate any “recovery” of the tissues did not result in any loss in mitochondrial viability (data not shown).

Provided that exposure times in excess of 40 h extend the experimental procedure duration beyond the standard timeline, it was determined that the fuels would be evaluated by a 24 h exposure to the tissues (six times longer than dermal exposure using the standard rabbit model) in order to accomplish a cross-comparison of the fuels. Tissues were exposed to 50 µl of each provided fuel (in triplicate) for 24 h. While JP-8 did not reduce mitochondrial viability of the tissues, exposure to three of the alternative fuels resulted in a loss in mitochondrial viability of 50% or greater (Figure 18).

Culture media collected immediately following the 24 h exposure to the fuels was evaluated for levels of IL-1α, an epidermal cytokine. Levels of IL-1α was significantly increased in the media from tissues exposed to 8 out of the 12 fuels tested, including JP-8 (but not S-8) and the three fuels that resulted in reduced mitochondrial viability (Figure 19). This suggests that IL-1α may serve as a marker of epidermal damage or stress due to irritation in this *in vitro* model.

As an alternative to the 3-dimensional human skin equivalent model, an adult human epidermal keratinocyte (HEKa) cell line was evaluated for effects on mitochondrial viability following fuel exposure. HEKa cells were isolated from human skin and were cryopreserved at the end of the primary culture stage (i.e., non-transformed line). This approach entailed a very short duration of exposure (10 min), followed by recovery periods of 0, 4, 8 and 24 h prior to determination of mitochondrial viability. After 4 h, 7 out of the 11 fuels tested displayed a significant reduction in mitochondrial viability, however, the majority of the viabilities remained >50% (Figure 5).

Time points other than 4 h displayed much more drastic reductions in viabilities that were difficult to compare (data not shown).

Taken together these dermal methods, the 3-dimensional human skin equivalent model and the human epidermal keratinocyte cells, provide two independent means for assessing fuel effects. The complexity arises from the difficulty of mimicking the slight to moderate irritancy effect of JP-8, as the human dermal models appear to be more robust towards JP-8 exposure than the rabbit. It should be considered that, although researchers have accepted the rabbit model, it may not be the most appropriate to predict human exposure to jet fuel. Additional studies are required to further assess the appropriateness of the *in vitro* models described here in order to validate them against other known irritants of a similar category to JP-8. Furthermore, other endpoints aside from mitochondrial viability and IL-1α may further strengthen these models.

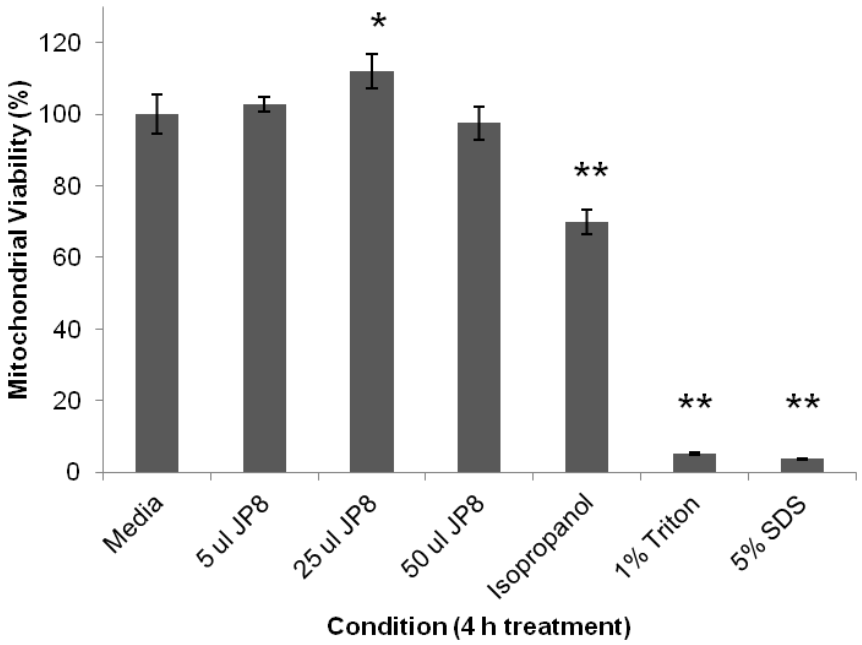


Figure 16. Mitochondrial viability of EpiDerm 3-D tissues following 4 h of exposure to increasing volumes of JP-8. *, p-value <0.05, **, p-value <0.0001 in comparison to media control.

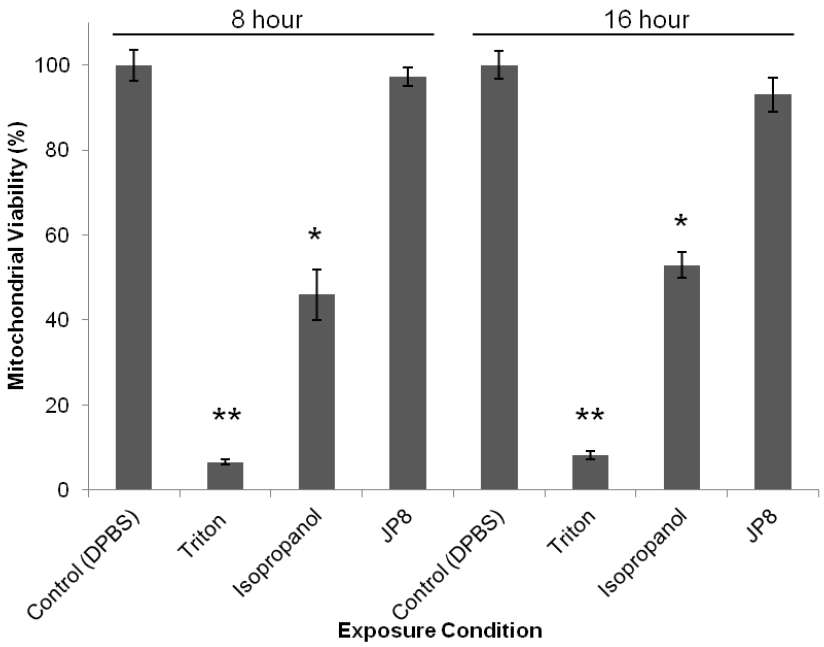


Figure 17. Mitochondrial viability of EpiDerm 3-D tissues following 8 and 16 h of exposure to 50 µl of JP-8. *, p-value <0.05, **, p-value <0.0001 in comparison to buffer control.

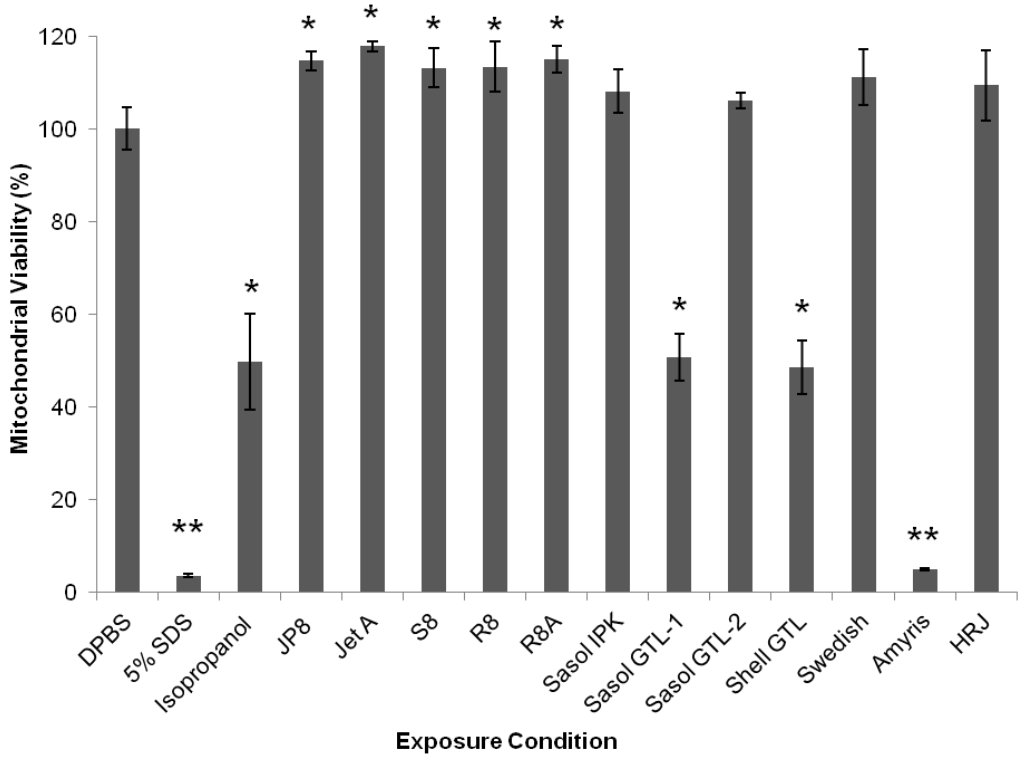


Figure 18. Mitochondrial viability of EpiDerm 3-D tissues following 24 h of exposure to 50 µl of jet fuel. *, p-value <0.05, **, p-value <0.0001 in comparison to buffer control.

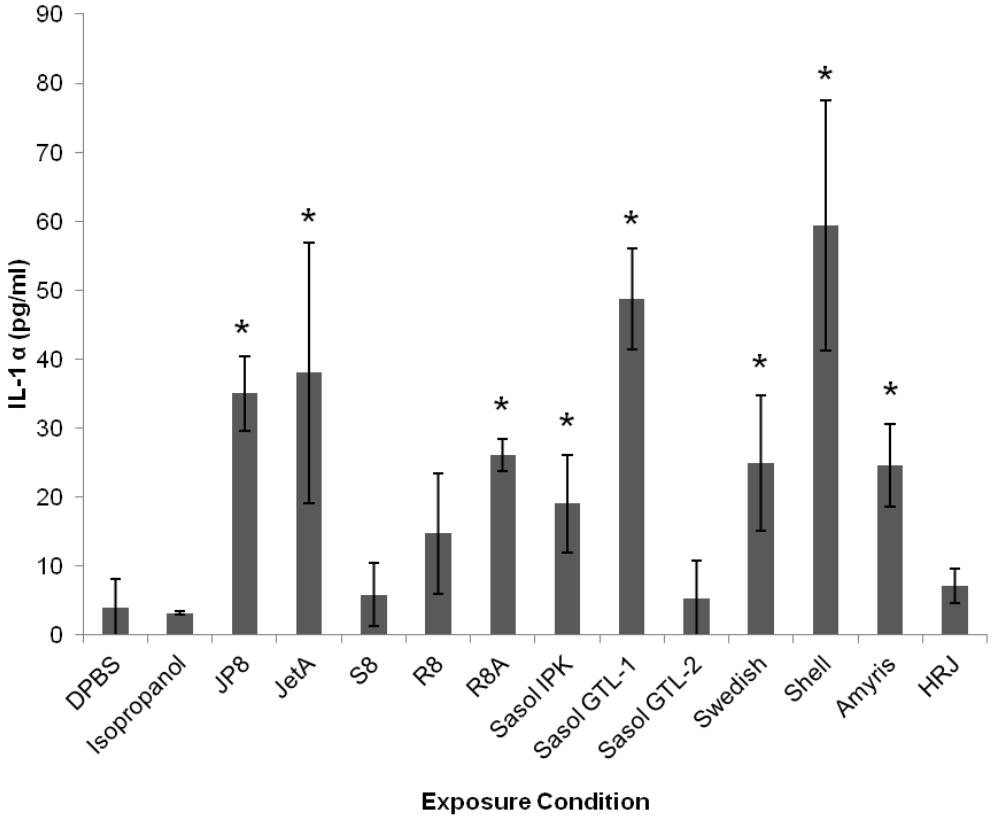


Figure 19. Production and release of IL-1α from EpiDerm 3-D tissues following 24 h of exposure to 50 µl of jet fuel.. *, p-value <0.05 in comparison to buffer control.

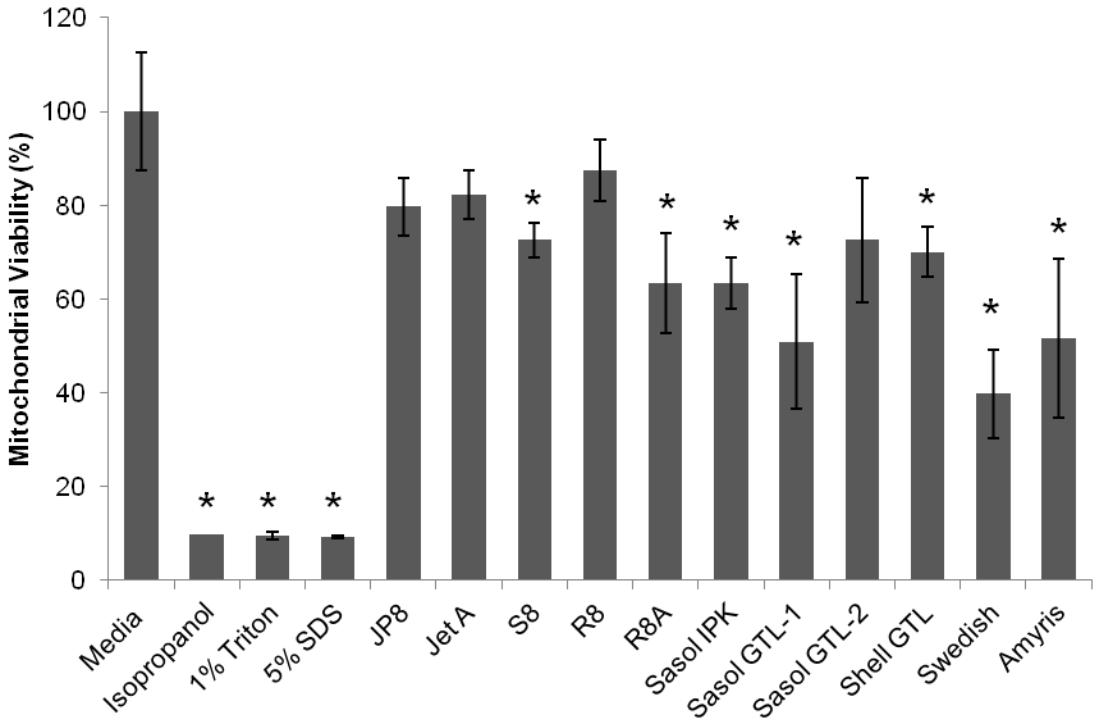


Figure 20. Mitochondrial viability of HEKa cells following 10 min exposure to jet fuel and 4 h recovery period.*, p-value <0.05 in comparison to media control.

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Kandarova H, Hayden P, Klausner M, Kubilus J, and Sheasgreen J. An *In Vitro* Skin Irritation Test (SIT) using the EpiDerm Reconstructed Human Epidermal (RHE) Model. *JoVE*. 29 (2009).

Mattie, D.R., Hinz, J.P., Wagner, D.J., Reddy, G., Steup, D.R., Wong, B.A. and Zeiger, E. Toxicity and Health Hazard Assessment for Synthetic Paraffinic Kerosene. *The Toxicologist*, 114(1): 220 (2010).

Wang S, Young RS, Sun NN, and Witten ML. Toxicology: In Vitro Cytokine Release From Type II Pneumocytes and Alveolar Macrophages Following Exposure to JP-8 Jet Fuel in Co-Culture. *Toxicology* 2002, 173, 211-219].

Overall Conclusions

In vitro techniques offer promise for predicting potential effects in humans. Pro-inflammatory cytokines may serve as biomarkers of effect but more research is necessary to determine which ones are optimal to include in cell-based or in vivo models. A lung co culture including human lung and immune cells showed promise as a means of rapidly assessing fuel toxicity and effects on the lung. However, further developments focusing on refining the physiological relevance of the lung model and developing a system in order to control and quantify the jet fuel vapor/aerosol exposures is required. The three dimensional human skin equivalent model did not work as a screen for dermal irritation. However a human epidermal keratinocyte cell model appears to work for alternative jet fuels but additional refinement is needed to complete the development.

Appendix A. Raw Data: Individual calculated concentrations for each cytokine by individual serum sample (Tables A-1 & A-2)

Table A-1: Concentrations (pg/ml) of IFN-γ, IL-13, IL-1β, and IL-4 by individual serum sample.

Plate_*29A7YA90331*													
Sample	Assay	Calc. Conc. Mean	Ave (pg/ml)	St Dev				Sample	Assay	Calc. Conc. Mean	Ave (pg/ml)	St Dev	
IFN-γ (Rat)								**IFN-γ (Rat)**					
701 Control male	IFN-γ (Rat)	6.97						801 Control female	IFN-γ (Rat)	12.62			
702 Control male	IFN-γ (Rat)	7.94						802 Control female	IFN-γ (Rat)	9.17			
703 Control male	IFN-γ (Rat)	9.01	7.95	1.01				803 Control female	IFN-γ (Rat)	6.57	10.80	2.91	
704 Control male	IFN-γ (Rat)	8.90						804 Control female	IFN-γ (Rat)	13.76			
705 Control male	IFN-γ (Rat)	6.92						805 Control female	IFN-γ (Rat)	11.87			
711 Medium male	IFN-γ (Rat)	9.33						811 Medium female	IFN-γ (Rat)	19.99			
712 Medium male	IFN-γ (Rat)	10.51						812 Medium female	IFN-γ (Rat)	7.75			
713 Medium male	IFN-γ (Rat)	10.32	9.85	0.71				813 Medium female	IFN-γ (Rat)	11.57	9.86	6.34	
714 Medium male	IFN-γ (Rat)	8.87						814 Medium female	IFN-γ (Rat)	3.73			
715 Medium male	IFN-γ (Rat)	10.21						815 Medium female	IFN-γ (Rat)	6.25			
716 High male	IFN-γ (Rat)	8.42						816 High female	IFN-γ (Rat)	4.75			
717 High male	IFN-γ (Rat)	8.93						817 High female	IFN-γ (Rat)	2.68			
718 High male	IFN-γ (Rat)	8.12	9.41	1.73				818 High female	IFN-γ (Rat)	4.48	4.55	2.68	
719 High male	IFN-γ (Rat)	12.43						819 High female	IFN-γ (Rat)	8.85			
720 High male	IFN-γ (Rat)	9.17						820 High female	IFN-γ (Rat)	1.98			
IL-13 (Rat)								**IL-13 (Rat)**					
701 Control male	IL-13 (Rat)	18.38						801 Control female	IL-13 (Rat)	19.37			
702 Control male	IL-13 (Rat)	18.83						802 Control female	IL-13 (Rat)	17.75			
703 Control male	IL-13 (Rat)	20.34	18.55	1.30				803 Control female	IL-13 (Rat)	17.77	18.48	0.80	
704 Control male	IL-13 (Rat)	16.69						804 Control female	IL-13 (Rat)	18.22			
705 Control male	IL-13 (Rat)	18.49						805 Control female	IL-13 (Rat)	19.30			
711 Medium male	IL-13 (Rat)	19.16						811 Medium female	IL-13 (Rat)	20.84			
712 Medium male	IL-13 (Rat)	21.31						812 Medium female	IL-13 (Rat)	23.38			
713 Medium male	IL-13 (Rat)	26.53	20.11	4.11				813 Medium female	IL-13 (Rat)	29.03	19.86	6.94	
714 Medium male	IL-13 (Rat)	17.69						814 Medium female	IL-13 (Rat)	13.91			
715 Medium male	IL-13 (Rat)	15.86						815 Medium female	IL-13 (Rat)	12.13			
716 High male	IL-13 (Rat)	14.62						816 High female	IL-13 (Rat)	14.86			
717 High male	IL-13 (Rat)	16.64						817 High female	IL-13 (Rat)	14.78			
718 High male	IL-13 (Rat)	19.51	18.03	2.76				818 High female	IL-13 (Rat)	15.85	16.53	2.16	
719 High male	IL-13 (Rat)	17.57						819 High female	IL-13 (Rat)	17.20			
720 High male	IL-13 (Rat)	21.83						820 High female	IL-13 (Rat)	19.98			
IL-1β (Rat)								**IL-1β (Rat)**					
701 Control male	IL-1β (Rat)	43.76						801 Control female	IL-1β (Rat)	20.71			
702 Control male	IL-1β (Rat)	26.25						802 Control female	IL-1β (Rat)	19.74			
703 Control male	IL-1β (Rat)	32.89	30.41	8.25				803 Control female	IL-1β (Rat)	17.26	19.90	2.41	
704 Control male	IL-1β (Rat)	25.76						804 Control female	IL-1β (Rat)	23.50			
705 Control male	IL-1β (Rat)	23.41						805 Control female	IL-1β (Rat)	18.29			
711 Medium male	IL-1β (Rat)	28.12						811 Medium female	IL-1β (Rat)	48.11			
712 Medium male	IL-1β (Rat)	25.14						812 Medium female	IL-1β (Rat)	22.24			
713 Medium male	IL-1β (Rat)	26.41	30.20	5.60				813 Medium female	IL-1β (Rat)	22.17	23.78	14.42	
714 Medium male	IL-1β (Rat)	38.93						814 Medium female	IL-1β (Rat)	10.87			
715 Medium male	IL-1β (Rat)	32.40						815 Medium female	IL-1β (Rat)	15.51			
716 High male	IL-1β (Rat)	34.11						816 High female	IL-1β (Rat)	13.75			
717 High male	IL-1β (Rat)	23.32						817 High female	IL-1β (Rat)	15.09			
718 High male	IL-1β (Rat)	38.54	32.44	7.41				818 High female	IL-1β (Rat)	13.69	15.90	2.52	
719 High male	IL-1β (Rat)	40.03						819 High female	IL-1β (Rat)	19.45			
720 High male	IL-1β (Rat)	26.20						820 High female	IL-1β (Rat)	17.54			
IL-4 (Rat)								**IL-4 (Rat)**					
701 Control male	IL-4 (Rat)	3.94						801 Control female	IL-4 (Rat)	3.93			
702 Control male	IL-4 (Rat)	1.86						802 Control female	IL-4 (Rat)	0.38			
703 Control male	IL-4 (Rat)	0.34	2.30	1.47				803 Control female	IL-4 (Rat)	0.30	3.11	2.63	
704 Control male	IL-4 (Rat)	1.78						804 Control female	IL-4 (Rat)	4.90			
705 Control male	IL-4 (Rat)	3.60						805 Control female	IL-4 (Rat)	6.02			
711 Medium male	IL-4 (Rat)	3.72						811 Medium female	IL-4 (Rat)	6.99			
712 Medium male	IL-4 (Rat)	5.24						812 Medium female	IL-4 (Rat)	1.32			
713 Medium male	IL-4 (Rat)	3.59	4.33	0.71				813 Medium female	IL-4 (Rat)	2.81	3.00	2.41	
714 Medium male	IL-4 (Rat)	4.83						814 Medium female	IL-4 (Rat)	0.90			
715 Medium male	IL-4 (Rat)	4.27						815 Medium female	IL-4 (Rat)	2.96			
716 High male	IL-4 (Rat)	4.97						816 High female	IL-4 (Rat)	1.61			
717 High male	IL-4 (Rat)	5.26						817 High female	IL-4 (Rat)	0.00			
718 High male	IL-4 (Rat)	1.26	4.31	1.72				818 High female	IL-4 (Rat)	0.90	1.21	1.33	
719 High male	IL-4 (Rat)	4.85						819 High female	IL-4 (Rat)	3.31			
720 High male	IL-4 (Rat)	5.24						820 High female	IL-4 (Rat)	0.24			

Note: Highlighted samples correspond to animals for which significant pathology was observed.

Table A-2: Concentrations (pg/ml) of IL-5, IL-8, and TNF-α by individual serum sample.

Plate_*29A7YA90331*													
Sample	Assay	Calc. Conc. Mean	Ave	St Dev				Sample	Assay	Calc. Conc. Mean	Ave	St Dev
			(pg/ml)								(pg/ml)	
IL-5 (Rat)								**IL-5 (Rat)**				
701 Control male	IL-5 (Rat)	155.17						801 Control female	IL-5 (Rat)	123.98		
702 Control male	IL-5 (Rat)	153.93						802 Control female	IL-5 (Rat)	93.35		
703 Control male	IL-5 (Rat)	176.03	153.59	14.38				803 Control female	IL-5 (Rat)	114.77	118.19	15.45
704 Control male	IL-5 (Rat)	144.88						804 Control female	IL-5 (Rat)	124.93		
705 Control male	IL-5 (Rat)	137.95						805 Control female	IL-5 (Rat)	133.91		
711 Medium male	IL-5 (Rat)	134.58						811 Medium female	IL-5 (Rat)	181.40		
712 Medium male	IL-5 (Rat)	149.79						812 Medium female	IL-5 (Rat)	82.05		
713 Medium male	IL-5 (Rat)	125.61	151.04	22.86				813 Medium female	IL-5 (Rat)	90.35	111.38	42.95
714 Medium male	IL-5 (Rat)	183.59						814 Medium female	IL-5 (Rat)	79.41		
715 Medium male	IL-5 (Rat)	161.62						815 Medium female	IL-5 (Rat)	123.70		
716 High male	IL-5 (Rat)	168.51						816 High female	IL-5 (Rat)	83.03		
717 High male	IL-5 (Rat)	144.64						817 High female	IL-5 (Rat)	75.63		
718 High male	IL-5 (Rat)	126.92	149.47	15.35				818 High female	IL-5 (Rat)	75.98	85.49	19.53
719 High male	IL-5 (Rat)	151.09						819 High female	IL-5 (Rat)	119.78		
720 High male	IL-5 (Rat)	156.19						820 High female	IL-5 (Rat)	73.03		
IL-8 (Rat)								**IL-8 (Rat)**				
701 Control male	IL-8 (Rat)	74.49						801 Control female	IL-8 (Rat)	95.91		
702 Control male	IL-8 (Rat)	107.61						802 Control female	IL-8 (Rat)	141.04		
703 Control male	IL-8 (Rat)	163.57	92.42	44.79				803 Control female	IL-8 (Rat)	195.40	119.87	48.73
704 Control male	IL-8 (Rat)	64.19						804 Control female	IL-8 (Rat)	91.35		
705 Control male	IL-8 (Rat)	52.24						805 Control female	IL-8 (Rat)	75.63		
711 Medium male	IL-8 (Rat)	205.21						811 Medium female	IL-8 (Rat)	196.00		
712 Medium male	IL-8 (Rat)	69.46						812 Medium female	IL-8 (Rat)	199.78		
713 Medium male	IL-8 (Rat)	82.35	95.37	62.68				813 Medium female	IL-8 (Rat)	202.95	174.43	43.61
714 Medium male	IL-8 (Rat)	72.08						814 Medium female	IL-8 (Rat)	174.41		
715 Medium male	IL-8 (Rat)	47.74						815 Medium female	IL-8 (Rat)	99.01		
716 High male	IL-8 (Rat)	53.04						816 High female	IL-8 (Rat)	128.49		
717 High male	IL-8 (Rat)	31.60						817 High female	IL-8 (Rat)	212.68		
718 High male	IL-8 (Rat)	240.68	90.00	86.16				818 High female	IL-8 (Rat)	178.47	162.05	46.21
719 High male	IL-8 (Rat)	43.89						819 High female	IL-8 (Rat)	100.46		
720 High male	IL-8 (Rat)	80.79						820 High female	IL-8 (Rat)	190.13		
TNF-α (Rat)								**TNF-α (Rat)**				
701 Control male	TNF-α (Rat)	335.08						801 Control female	TNF-α (Rat)	455.87		
702 Control male	TNF-α (Rat)	361.47						802 Control female	TNF-α (Rat)	434.92		
703 Control male	TNF-α (Rat)	398.10	364.62	22.50				803 Control female	TNF-α (Rat)	426.59	436.44	23.05
704 Control male	TNF-α (Rat)	367.42						804 Control female	TNF-α (Rat)	403.99		
705 Control male	TNF-α (Rat)	361.02						805 Control female	TNF-α (Rat)	460.85		
711 Medium male	TNF-α (Rat)	455.55						811 Medium female	TNF-α (Rat)	459.10		
712 Medium male	TNF-α (Rat)	356.46						812 Medium female	TNF-α (Rat)	455.24		
713 Medium male	TNF-α (Rat)	330.27	381.88	46.81				813 Medium female	TNF-α (Rat)	409.35	390.70	72.83
714 Medium male	TNF-α (Rat)	386.97						814 Medium female	TNF-α (Rat)	298.53		
715 Medium male	TNF-α (Rat)	380.15						815 Medium female	TNF-α (Rat)	331.27		
716 High male	TNF-α (Rat)	384.87						816 High female	TNF-α (Rat)	382.17		
717 High male	TNF-α (Rat)	411.23						817 High female	TNF-α (Rat)	378.00		
718 High male	TNF-α (Rat)	454.17	411.32	27.44				818 High female	TNF-α (Rat)	390.91	372.52	16.25
719 High male	TNF-α (Rat)	390.09						819 High female	TNF-α (Rat)	351.67		
720 High male	TNF-α (Rat)	416.23						820 High female	TNF-α (Rat)	359.86		

Note: Highlighted samples correspond to animals for which significant pathology was observed.

Appendix B. Raw Data: Individual calculated concentrations measured in the media for each cytokine by individual sample (Tables B-1 to B-14)

Table B-1: Calculated concentrations (pg/ml) of GM-CSF measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: GM-CSF (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	1009	1013	0.558	5.566	5.594	0.693
Amyris 100 ug/ml 1	1017	1013	0.558	5.621	5.594	0.693
Amyris 100 ug/ml 2	1054	1008	6.454	5.875	5.560	8.017
Amyris 100 ug/ml 2	962	1008	6.454	5.245	5.560	8.017
Amyris 100 ug/ml 3	1149	1147	0.308	6.531	6.513	0.376
Amyris 100 ug/ml 3	1144	1147	0.308	6.496	6.513	0.376
JP8 10 ug/ml 1	948	965	2.419	5.149	5.262	3.028
JP8 10 ug/ml 1	981	965	2.419	5.375	5.262	3.028
JP8 10 ug/ml 2	1231	1155	9.306	7.100	6.574	11.321
JP8 10 ug/ml 2	1079	1155	9.306	6.047	6.574	11.321
JP8 10 ug/ml 3	844	834	1.782	4.443	4.373	2.292
JP8 10 ug/ml 3	823	834	1.782	4.302	4.373	2.292
JP8 100 ug/ml 1	911	895	2.528	4.898	4.789	3.207
JP8 100 ug/ml 1	879	895	2.528	4.680	4.789	3.207
JP8 100 ug/ml 2	842	811	5.406	4.430	4.221	6.995
JP8 100 ug/ml 2	780	811	5.406	4.012	4.221	6.995
JP8 100 ug/ml 3	882	894	1.898	4.701	4.782	2.409
JP8 100 ug/ml 3	906	894	1.898	4.864	4.782	2.409
JP8 100 ug/ml 4	938	933	0.834	5.081	5.044	1.050
JP8 100 ug/ml 4	927	933	0.834	5.006	5.044	1.050
JP8 C1	971	982	1.513	5.306	5.378	1.888
JP8 C1	992	982	1.513	5.450	5.378	1.888
JP8 C2	818	845	4.438	4.268	4.447	5.694
JP8 C2	871	845	4.438	4.626	4.447	5.694
JP8 C4	810	800	1.768	4.214	4.147	2.294
JP8 C4	790	800	1.768	4.080	4.147	2.294
JP8 DMSO 1	873	865	1.390	4.640	4.582	1.776
JP8 DMSO 1	856	865	1.390	4.525	4.582	1.776
JP8 DMSO 3	929	893	5.784	5.020	4.772	7.341
JP8 DMSO 3	856	893	5.784	4.525	4.772	7.341
JP8 DMSO 4	963	1000	5.233	5.252	5.505	6.509
JP8 DMSO 4	1037	1000	5.233	5.759	5.505	6.509
Media	298	287	5.420	0.891	0.824	11.475
Media	276	287	5.420	0.757	0.824	11.475
R8 100 2	1333	1303	3.312	7.812	7.599	3.966
R8 100 2	1272	1303	3.312	7.386	7.599	3.966
R8 100 ug/ml 3	975	1000	3.467	5.334	5.502	4.313
R8 100 ug/ml 3	1024	1000	3.467	5.669	5.502	4.313
R8 100 ug/ml 4	1129	1116	1.647	6.392	6.303	2.015
R8 100 ug/ml 4	1103	1116	1.647	6.213	6.303	2.015
R8 C2	1025	983	6.117	5.676	5.386	7.632
R8 C2	940	983	6.117	5.095	5.386	7.632
R8 C3	932	862	11.573	5.040	4.563	14.785
R8 C3	791	862	11.573	4.086	4.563	14.785
R8 C4	951	909	6.534	5.170	4.884	8.265
R8 C4	867	909	6.534	4.599	4.884	8.265
R8 DMSO 2	848	868	3.259	4.470	4.606	4.158
R8 DMSO 2	888	868	3.259	4.741	4.606	4.158
R8 DMSO 3	856	845	1.841	4.525	4.450	2.362
R8 DMSO 3	834	845	1.841	4.376	4.450	2.362
R8 DMSO 4	925	888	5.976	4.993	4.738	7.592
R8 DMSO 4	850	888	5.976	4.484	4.738	7.592

Table B-1 con't: Calculated concentrations (pg/ml) of GM-CSF measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: GM-CSF (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	1029	998	4.466	5.704	5.488	5.558
S8 100 ug/ml 1	966	998	4.466	5.272	5.488	5.558
S8 100 ug/ml 2	890	895	0.790	4.755	4.789	1.002
S8 100 ug/ml 2	900	895	0.790	4.823	4.789	1.002
S8 100 ug/ml 3	842	907	10.135	4.430	4.872	12.822
S8 100 ug/ml 3	972	907	10.135	5.313	4.872	12.822
S8 100 ug/ml 4	965	1051	11.510	5.265	5.853	14.199
S8 100 ug/ml 4	1136	1051	11.510	6.441	5.853	14.199
S8 C1	1105	1397	29.520	6.227	8.273	34.981
S8 C1	1688	1397	29.520	10.319	8.273	34.981
S8 C2	994	977	2.534	5.464	5.344	3.165
S8 C2	959	977	2.534	5.225	5.344	3.165
S8 C4	1175	1197	2.599	6.711	6.864	3.147
S8 C4	1219	1197	2.599	7.016	6.864	3.147
S8 DMSO 1	820	817	0.606	4.281	4.258	0.783
S8 DMSO 1	813	817	0.606	4.234	4.258	0.783
S8 DMSO 2	929	949	2.980	5.020	5.156	3.741
S8 DMSO 2	969	949	2.980	5.293	5.156	3.741
S8 DMSO 3	802	839	6.237	4.160	4.410	8.013
S8 DMSO 3	876	839	6.237	4.660	4.410	8.013
Swedish 100 ug/ml 1	1093	1094	0.129	6.144	6.151	0.159
Swedish 100 ug/ml 1	1095	1094	0.129	6.158	6.151	0.159
Swedish 100 ug/ml 2	1040	1078	4.922	5.779	6.037	6.050
Swedish 100 ug/ml 2	1115	1078	4.922	6.296	6.037	6.050
Swedish 100 ug/ml 3	965	1005	5.629	5.265	5.539	6.996
Swedish 100 ug/ml 3	1045	1005	5.629	5.814	5.539	6.996
Swedish 100 ug/ml 4	1262	1318	5.957	7.316	7.704	7.125
Swedish 100 ug/ml 4	1373	1318	5.957	8.092	7.704	7.125

Table B-2: Calculated concentrations (pg/ml) of IL-1β measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-1β (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	1705	1655	4.273	4.159	3.990	5.998
Amyris 100 ug/ml 1	1605	1655	4.273	3.820	3.990	5.998
Amyris 100 ug/ml 2	1471	1521	4.649	3.367	3.536	6.773
Amyris 100 ug/ml 2	1571	1521	4.649	3.705	3.536	6.773
Amyris 100 ug/ml 3	1746	1836	6.932	4.298	4.602	9.353
Amyris 100 ug/ml 3	1926	1836	6.932	4.906	4.602	9.353
JP8 10 ug/ml 1	1215	1233	2.065	2.499	2.560	3.374
JP8 10 ug/ml 1	1251	1233	2.065	2.621	2.560	3.374
JP8 10 ug/ml 2	1576	1493	7.862	3.722	3.441	11.554
JP8 10 ug/ml 2	1410	1493	7.862	3.160	3.441	11.554
JP8 10 ug/ml 3	1120	1066	7.234	2.176	1.991	13.155
JP8 10 ug/ml 3	1011	1066	7.234	1.806	1.991	13.155
JP8 100 ug/ml 1	1204	1165	4.734	2.461	2.329	8.040
JP8 100 ug/ml 1	1126	1165	4.734	2.197	2.329	8.040
JP8 100 ug/ml 2	1020	1037	2.251	1.836	1.892	4.191
JP8 100 ug/ml 2	1053	1037	2.251	1.949	1.892	4.191
JP8 100 ug/ml 3	1212	1215	0.291	2.489	2.497	0.480
JP8 100 ug/ml 3	1217	1215	0.291	2.505	2.497	0.480
JP8 100 ug/ml 4	1193	1175	2.166	2.424	2.363	3.657
JP8 100 ug/ml 4	1157	1175	2.166	2.302	2.363	3.657
JP8 C1	1490	1489	0.143	3.431	3.426	0.210
JP8 C1	1487	1489	0.143	3.421	3.426	0.210
JP8 C2	1006	1021	2.009	1.789	1.838	3.793
JP8 C2	1035	1021	2.009	1.887	1.838	3.793
JP8 C4	906	876	4.843	1.449	1.346	10.734
JP8 C4	846	876	4.843	1.244	1.346	10.734
JP8 DMSO 1	1255	1261	0.617	2.634	2.653	0.995
JP8 DMSO 1	1266	1261	0.617	2.672	2.653	0.995
JP8 DMSO 3	1128	1133	0.562	2.203	2.219	0.974
JP8 DMSO 3	1137	1133	0.562	2.234	2.219	0.974
JP8 DMSO 4	1200	1247	5.330	2.448	2.607	8.649
JP8 DMSO 4	1294	1247	5.330	2.767	2.607	8.649
Media	271	286	7.183	0.000	0.000	NaN
Media	300	286	7.183	0.000	0.000	NaN
R8 100 2	2067	2086	1.288	5.383	5.447	1.667
R8 100 2	2105	2086	1.288	5.511	5.447	1.667
R8 100 ug/ml 3	1371	1346	2.627	3.028	2.943	4.073
R8 100 ug/ml 3	1321	1346	2.627	2.858	2.943	4.073
R8 100 ug/ml 4	1634	1644	0.817	3.919	3.951	1.151
R8 100 ug/ml 4	1653	1644	0.817	3.983	3.951	1.151
R8 C2	1336	1302	3.749	2.909	2.792	5.926
R8 C2	1267	1302	3.749	2.675	2.792	5.926
R8 C3	968	1004	5.003	1.660	1.780	9.589
R8 C3	1039	1004	5.003	1.901	1.780	9.589
R8 C4	1235	1511	25.832	2.567	3.501	37.758
R8 C4	1787	1511	25.832	4.436	3.501	37.758
R8 DMSO 2	1064	1036	3.822	1.986	1.891	7.119
R8 DMSO 2	1008	1036	3.822	1.796	1.891	7.119
R8 DMSO 3	1146	1136	1.245	2.264	2.231	2.153
R8 DMSO 3	1126	1136	1.245	2.197	2.231	2.153
R8 DMSO 4	1091	1066	3.385	2.078	1.991	6.155
R8 DMSO 4	1040	1066	3.385	1.904	1.991	6.155

Table B- 2 con't: Calculated concentrations (pg/ml) of IL-1β measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-1β (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	1638	1678	3.371	3.932	4.067	4.706
S8 100 ug/ml 1	1718	1678	3.371	4.203	4.067	4.706
S8 100 ug/ml 2	1704	1717	1.030	4.155	4.198	1.425
S8 100 ug/ml 2	1729	1717	1.030	4.240	4.198	1.425
S8 100 ug/ml 3	1381	1265	12.968	3.062	2.668	20.857
S8 100 ug/ml 3	1149	1265	12.968	2.275	2.668	20.857
S8 100 ug/ml 4	1801	1742	4.790	4.484	4.284	6.589
S8 100 ug/ml 4	1683	1742	4.790	4.084	4.284	6.589
S8 C1	1912	1924	0.882	4.859	4.900	1.171
S8 C1	1936	1924	0.882	4.940	4.900	1.171
S8 C2	1434	1430	0.396	3.241	3.228	0.594
S8 C2	1426	1430	0.396	3.214	3.228	0.594
S8 C4	1905	1972	4.805	4.835	5.062	6.327
S8 C4	2039	1972	4.805	5.288	5.062	6.327
S8 DMSO 1	1227	1232	0.517	2.539	2.555	0.845
S8 DMSO 1	1236	1232	0.517	2.570	2.555	0.845
S8 DMSO 2	1524	1560	3.219	3.546	3.666	4.637
S8 DMSO 2	1595	1560	3.219	3.787	3.666	4.637
S8 DMSO 3	1203	1170	4.051	2.458	2.344	6.861
S8 DMSO 3	1136	1170	4.051	2.231	2.344	6.861
Swedish 100 ug/ml 1	1647	1567	7.220	3.963	3.692	10.376
Swedish 100 ug/ml 1	1487	1567	7.220	3.421	3.692	10.376
Swedish 100 ug/ml 2	1567	1532	3.278	3.692	3.572	4.760
Swedish 100 ug/ml 2	1496	1532	3.278	3.451	3.572	4.760
Swedish 100 ug/ml 3	1679	1809	10.127	4.071	4.509	13.738
Swedish 100 ug/ml 3	1938	1809	10.127	4.947	4.509	13.738
Swedish 100 ug/ml 4	2371	2438	3.858	6.410	6.634	4.785
Swedish 100 ug/ml 4	2504	2438	3.858	6.859	6.634	4.785

Table B-3: Calculated concentrations (pg/ml) of IL-2 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-2 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	720	683	7.770	2.218	2.081	9.258
Amyris 100 ug/ml 1	645	683	7.770	1.945	2.081	9.258
Amyris 100 ug/ml 2	736	668	14.513	2.276	2.028	17.307
Amyris 100 ug/ml 2	599	668	14.513	1.780	2.028	17.307
Amyris 100 ug/ml 3	607	636	6.342	1.808	1.911	7.597
Amyris 100 ug/ml 3	664	636	6.342	2.014	1.911	7.597
JP8 10 ug/ml 1	553	582	7.047	1.616	1.719	8.502
JP8 10 ug/ml 1	611	582	7.047	1.823	1.719	8.502
JP8 10 ug/ml 2	618	609	2.208	1.848	1.814	2.654
JP8 10 ug/ml 2	599	609	2.208	1.780	1.814	2.654
JP8 10 ug/ml 3	677	634	9.711	2.061	1.904	11.633
JP8 10 ug/ml 3	590	634	9.711	1.748	1.904	11.633
JP8 100 ug/ml 1	630	611	4.398	1.891	1.823	5.285
JP8 100 ug/ml 1	592	611	4.398	1.755	1.823	5.285
JP8 100 ug/ml 2	487	510	6.378	1.384	1.465	7.790
JP8 100 ug/ml 2	533	510	6.378	1.545	1.465	7.790
JP8 100 ug/ml 3	526	525	0.269	1.521	1.517	0.328
JP8 100 ug/ml 3	524	525	0.269	1.514	1.517	0.328
JP8 100 ug/ml 4	584	625	9.171	1.726	1.872	11.000
JP8 100 ug/ml 4	665	625	9.171	2.017	1.872	11.000
JP8 C1	805	802	0.529	2.531	2.519	0.624
JP8 C1	799	802	0.529	2.508	2.519	0.624
JP8 C2	604	624	4.533	1.798	1.870	5.438
JP8 C2	644	624	4.533	1.942	1.870	5.438
JP8 C4	489	500	2.973	1.391	1.428	3.639
JP8 C4	510	500	2.973	1.464	1.428	3.639
JP8 DMSO 1	558	558	0.000	1.634	1.634	0.000
JP8 DMSO 1	558	558	0.000	1.634	1.634	0.000
JP8 DMSO 3	572	569	0.746	1.684	1.673	0.902
JP8 DMSO 3	566	569	0.746	1.662	1.673	0.902
JP8 DMSO 4	1636	1154	59.155	5.756	3.898	67.434
JP8 DMSO 4	671	1154	59.155	2.039	3.898	67.434
Media	239	279	20.058	0.552	0.681	26.647
Media	318	279	20.058	0.809	0.681	26.647
R8 100 2	732	752	3.761	2.262	2.335	4.452
R8 100 2	772	752	3.761	2.409	2.335	4.452
R8 100 ug/ml 3	659	707	9.601	1.996	2.171	11.409
R8 100 ug/ml 3	755	707	9.601	2.346	2.171	11.409
R8 100 ug/ml 4	592	617	5.730	1.755	1.845	6.881
R8 100 ug/ml 4	642	617	5.730	1.934	1.845	6.881
R8 C2	540	528	3.214	1.570	1.528	3.913
R8 C2	516	528	3.214	1.486	1.528	3.913
R8 C3	677	645	7.016	2.061	1.945	8.395
R8 C3	613	645	7.016	1.830	1.945	8.395
R8 C4	709	686	4.742	2.177	2.094	5.648
R8 C4	663	686	4.742	2.010	2.094	5.648
R8 DMSO 2	545	540	1.442	1.588	1.568	1.752
R8 DMSO 2	534	540	1.442	1.549	1.568	1.752
R8 DMSO 3	532	528	1.071	1.542	1.528	1.304
R8 DMSO 3	524	528	1.071	1.514	1.528	1.304
R8 DMSO 4	466	475	2.533	1.311	1.341	3.118
R8 DMSO 4	483	475	2.533	1.370	1.341	3.118

Table B-3 con't: Calculated concentrations (pg/ml) of IL-2 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-2 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	613	632	4.143	1.830	1.897	4.966
S8 100 ug/ml 1	650	632	4.143	1.963	1.897	4.966
S8 100 ug/ml 2	540	519	5.722	1.570	1.496	6.977
S8 100 ug/ml 2	498	519	5.722	1.422	1.496	6.977
S8 100 ug/ml 3	489	497	2.276	1.391	1.419	2.788
S8 100 ug/ml 3	505	497	2.276	1.447	1.419	2.788
S8 100 ug/ml 4	550	563	3.266	1.605	1.652	3.952
S8 100 ug/ml 4	576	563	3.266	1.698	1.652	3.952
S8 C1	758	745	2.468	2.357	2.309	2.923
S8 C1	732	745	2.468	2.262	2.309	2.923
S8 C2	565	591	6.222	1.659	1.751	7.497
S8 C2	617	591	6.222	1.844	1.751	7.497
S8 C4	719	738	3.548	2.214	2.282	4.205
S8 C4	756	738	3.548	2.350	2.282	4.205
S8 DMSO 1	544	557	3.301	1.584	1.630	3.998
S8 DMSO 1	570	557	3.301	1.676	1.630	3.998
S8 DMSO 2	731	743	2.190	2.258	2.300	2.595
S8 DMSO 2	754	743	2.190	2.342	2.300	2.595
S8 DMSO 3	533	540	1.833	1.545	1.570	2.227
S8 DMSO 3	547	540	1.833	1.595	1.570	2.227
Swedish 100 ug/ml 1	626	622	1.024	1.877	1.860	1.229
Swedish 100 ug/ml 1	617	622	1.024	1.844	1.860	1.229
Swedish 100 ug/ml 2	780	713	13.398	2.438	2.192	15.905
Swedish 100 ug/ml 2	645	713	13.398	1.945	2.192	15.905
Swedish 100 ug/ml 3	594	654	12.974	1.762	1.979	15.499
Swedish 100 ug/ml 3	714	654	12.974	2.196	1.979	15.499
Swedish 100 ug/ml 4	832	842	1.680	2.631	2.668	1.975
Swedish 100 ug/ml 4	852	842	1.680	2.705	2.668	1.975

Table B-4: Calculated concentrations (pg/ml) of IL-5 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-5 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	377	378	0.187	0.000	0.000	NaN
Amyris 100 ug/ml 1	378	378	0.187	0.000	0.000	NaN
Amyris 100 ug/ml 2	394	365	11.236	0.000	0.000	NaN
Amyris 100 ug/ml 2	336	365	11.236	0.000	0.000	NaN
Amyris 100 ug/ml 3	467	468	0.151	0.225	0.227	1.137
Amyris 100 ug/ml 3	468	468	0.151	0.228	0.227	1.137
JP8 10 ug/ml 1	456	437	6.149	0.185	0.114	87.367
JP8 10 ug/ml 1	418	437	6.149	0.044	0.114	87.367
JP8 10 ug/ml 2	392	425	10.981	0.000	0.096	141.421
JP8 10 ug/ml 2	458	425	10.981	0.192	0.096	141.421
JP8 10 ug/ml 3	376	367	3.666	0.000	0.000	NaN
JP8 10 ug/ml 3	357	367	3.666	0.000	0.000	NaN
JP8 100 ug/ml 1	296	330	14.378	0.000	0.000	NaN
JP8 100 ug/ml 1	363	330	14.378	0.000	0.000	NaN
JP8 100 ug/ml 2	371	339	13.578	0.000	0.000	NaN
JP8 100 ug/ml 2	306	339	13.578	0.000	0.000	NaN
JP8 100 ug/ml 3	306	311	2.050	0.000	0.000	NaN
JP8 100 ug/ml 3	315	311	2.050	0.000	0.000	NaN
JP8 100 ug/ml 4	388	397	3.032	0.000	0.000	NaN
JP8 100 ug/ml 4	405	397	3.032	0.000	0.000	NaN
JP8 C1	458	437	6.796	0.192	0.114	96.732
JP8 C1	416	437	6.796	0.036	0.114	96.732
JP8 C2	342	323	8.551	0.000	0.000	NaN
JP8 C2	303	323	8.551	0.000	0.000	NaN
JP8 C4	322	333	4.672	0.000	0.000	NaN
JP8 C4	344	333	4.672	0.000	0.000	NaN
JP8 DMSO 1	347	323	10.744	0.000	0.000	NaN
JP8 DMSO 1	298	323	10.744	0.000	0.000	NaN
JP8 DMSO 3	372	365	2.712	0.000	0.000	NaN
JP8 DMSO 3	358	365	2.712	0.000	0.000	NaN
JP8 DMSO 4	359	361	0.588	0.000	0.000	NaN
JP8 DMSO 4	362	361	0.588	0.000	0.000	NaN
Media	208	217	5.865	0.000	0.000	NaN
Media	226	217	5.865	0.000	0.000	NaN
R8 100 2	447	475	8.336	0.152	0.253	56.809
R8 100 2	503	475	8.336	0.355	0.253	56.809
R8 100 ug/ml 3	450	436	4.709	0.163	0.109	69.974
R8 100 ug/ml 3	421	436	4.709	0.055	0.109	69.974
R8 100 ug/ml 4	387	375	4.720	0.000	0.000	NaN
R8 100 ug/ml 4	362	375	4.720	0.000	0.000	NaN
R8 C2	302	337	14.499	0.000	0.000	NaN
R8 C2	371	337	14.499	0.000	0.000	NaN
R8 C3	415	361	21.380	0.032	0.016	141.421
R8 C3	306	361	21.380	0.000	0.016	141.421
R8 C4	382	341	17.236	0.000	0.000	NaN
R8 C4	299	341	17.236	0.000	0.000	NaN
R8 DMSO 2	339	365	10.074	0.000	0.000	NaN
R8 DMSO 2	391	365	10.074	0.000	0.000	NaN
R8 DMSO 3	316	316	0.000	0.000	0.000	NaN
R8 DMSO 3	316	316	0.000	0.000	0.000	NaN
R8 DMSO 4	340	347	2.853	0.000	0.000	NaN
R8 DMSO 4	354	347	2.853	0.000	0.000	NaN

Table B-4 con't: Calculated concentrations (pg/ml) of IL-5 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-5 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	380	367	5.009	0.000	0.000	NaN
S8 100 ug/ml 1	354	367	5.009	0.000	0.000	NaN
S8 100 ug/ml 2	419	390	10.516	0.047	0.024	141.421
S8 100 ug/ml 2	361	390	10.516	0.000	0.024	141.421
S8 100 ug/ml 3	363	346	7.163	0.000	0.000	NaN
S8 100 ug/ml 3	328	346	7.163	0.000	0.000	NaN
S8 100 ug/ml 4	394	396	0.536	0.000	0.000	NaN
S8 100 ug/ml 4	397	396	0.536	0.000	0.000	NaN
S8 C1	399	425	8.652	0.000	0.083	141.421
S8 C1	451	425	8.652	0.166	0.083	141.421
S8 C2	405	386	7.154	0.000	0.000	NaN
S8 C2	366	386	7.154	0.000	0.000	NaN
S8 C4	653	543	28.649	0.889	0.494	112.851
S8 C4	433	543	28.649	0.100	0.494	112.851
S8 DMSO 1	340	362	8.595	0.000	0.000	NaN
S8 DMSO 1	384	362	8.595	0.000	0.000	NaN
S8 DMSO 2	324	354	11.802	0.000	0.000	NaN
S8 DMSO 2	383	354	11.802	0.000	0.000	NaN
S8 DMSO 3	409	379	11.396	0.009	0.004	141.421
S8 DMSO 3	348	379	11.396	0.000	0.004	141.421
Swedish 100 ug/ml 1	387	387	0.183	0.000	0.000	NaN
Swedish 100 ug/ml 1	386	387	0.183	0.000	0.000	NaN
Swedish 100 ug/ml 2	493	457	11.308	0.319	0.185	102.320
Swedish 100 ug/ml 2	420	457	11.308	0.051	0.185	102.320
Swedish 100 ug/ml 3	373	370	1.340	0.000	0.000	NaN
Swedish 100 ug/ml 3	366	370	1.340	0.000	0.000	NaN
Swedish 100 ug/ml 4	574	573	0.371	0.609	0.604	1.250
Swedish 100 ug/ml 4	571	573	0.371	0.599	0.604	1.250

Table B-5: Calculated concentrations (pg/ml) of IL-6 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-6 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	3532	3467	2.672	6.161	6.025	3.191
Amyris 100 ug/ml 1	3401	3467	2.672	5.890	6.025	3.191
Amyris 100 ug/ml 2	3230	3253	0.978	5.536	5.582	1.179
Amyris 100 ug/ml 2	3275	3253	0.978	5.629	5.582	1.179
Amyris 100 ug/ml 3	4794	4619	5.374	8.808	8.438	6.206
Amyris 100 ug/ml 3	4443	4619	5.374	8.067	8.438	6.206
JP8 10 ug/ml 1	2986	3021	1.615	5.032	5.103	1.969
JP8 10 ug/ml 1	3055	3021	1.615	5.174	5.103	1.969
JP8 10 ug/ml 2	4193	4398	6.577	7.542	7.972	7.633
JP8 10 ug/ml 2	4602	4398	6.577	8.402	7.972	7.633
JP8 10 ug/ml 3	2831	2701	6.807	4.714	4.448	8.456
JP8 10 ug/ml 3	2571	2701	6.807	4.182	4.448	8.456
JP8 100 ug/ml 1	3338	3205	5.869	5.759	5.484	7.086
JP8 100 ug/ml 1	3072	3205	5.869	5.209	5.484	7.086
JP8 100 ug/ml 2	2410	2385	1.482	3.854	3.803	1.888
JP8 100 ug/ml 2	2360	2385	1.482	3.753	3.803	1.888
JP8 100 ug/ml 3	2774	2649	6.701	4.597	4.340	8.356
JP8 100 ug/ml 3	2523	2649	6.701	4.084	4.340	8.356
JP8 100 ug/ml 4	3378	3352	1.097	5.842	5.788	1.316
JP8 100 ug/ml 4	3326	3352	1.097	5.734	5.788	1.316
JP8 C1	6382	6169	4.895	12.195	11.736	5.519
JP8 C1	5955	6169	4.895	11.278	11.736	5.519
JP8 C2	2357	2405	2.823	3.746	3.844	3.588
JP8 C2	2453	2405	2.823	3.941	3.844	3.588
JP8 C4	2268	2278	0.621	3.566	3.586	0.799
JP8 C4	2288	2278	0.621	3.606	3.586	0.799
JP8 DMSO 1	3282	3248	1.480	5.643	5.573	1.784
JP8 DMSO 1	3214	3248	1.480	5.502	5.573	1.784
JP8 DMSO 3	3091	2972	5.663	5.249	5.004	6.919
JP8 DMSO 3	2853	2972	5.663	4.759	5.004	6.919
JP8 DMSO 4	4381	4233	4.962	7.937	7.625	5.782
JP8 DMSO 4	4084	4233	4.962	7.313	7.625	5.782
Media	297	296	0.478	0.000	0.000	NaN
Media	295	296	0.478	0.000	0.000	NaN
R8 100 2	5874	5623	6.313	11.105	10.570	7.166
R8 100 2	5372	5623	6.313	10.034	10.570	7.166
R8 100 ug/ml 3	3216	3365	6.262	5.507	5.815	7.508
R8 100 ug/ml 3	3514	3365	6.262	6.124	5.815	7.508
R8 100 ug/ml 4	3318	3561	9.632	5.718	6.222	11.458
R8 100 ug/ml 4	3803	3561	9.632	6.726	6.222	11.458
R8 C2	3190	3271	3.502	5.453	5.620	4.216
R8 C2	3352	3271	3.502	5.788	5.620	4.216
R8 C3	2796	2608	10.194	4.642	4.258	12.747
R8 C3	2420	2608	10.194	3.874	4.258	12.747
R8 C4	3192	2862	16.306	5.457	4.780	20.041
R8 C4	2532	2862	16.306	4.102	4.780	20.041
R8 DMSO 2	2543	2542	0.083	4.125	4.122	0.105
R8 DMSO 2	2540	2542	0.083	4.119	4.122	0.105
R8 DMSO 3	2454	2523	3.840	3.944	4.083	4.834
R8 DMSO 3	2591	2523	3.840	4.223	4.083	4.834
R8 DMSO 4	2153	2259	6.606	3.333	3.547	8.515
R8 DMSO 4	2364	2259	6.606	3.761	3.547	8.515

Table B-5 con't: Calculated concentrations (pg/ml) of IL-6 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-6 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	5171	5169	0.068	9.607	9.601	0.078
S8 100 ug/ml 1	5166	5169	0.068	9.596	9.601	0.078
S8 100 ug/ml 2	2871	2857	0.693	4.796	4.767	0.852
S8 100 ug/ml 2	2843	2857	0.693	4.738	4.767	0.852
S8 100 ug/ml 3	3002	2805	9.959	5.065	4.660	12.288
S8 100 ug/ml 3	2607	2805	9.959	4.255	4.660	12.288
S8 100 ug/ml 4	3500	3593	3.661	6.095	6.288	4.350
S8 100 ug/ml 4	3686	3593	3.661	6.482	6.288	4.350
S8 C1	5310	5424	2.960	9.902	10.144	3.370
S8 C1	5537	5424	2.960	10.385	10.144	3.370
S8 C2	3295	3294	0.043	5.670	5.668	0.052
S8 C2	3293	3294	0.043	5.666	5.668	0.052
S8 C4	5321	5083	6.622	9.925	9.421	7.580
S8 C4	4845	5083	6.622	8.916	9.421	7.580
S8 DMSO 1	2548	2620	3.860	4.135	4.281	4.824
S8 DMSO 1	2691	2620	3.860	4.427	4.281	4.824
S8 DMSO 2	3764	3498	10.776	6.644	6.091	12.849
S8 DMSO 2	3231	3498	10.776	5.538	6.091	12.849
S8 DMSO 3	2561	2454	6.196	4.161	3.943	7.844
S8 DMSO 3	2346	2454	6.196	3.724	3.943	7.844
Swedish 100 ug/ml 1	2930	3156	10.106	4.917	5.383	12.230
Swedish 100 ug/ml 1	3381	3156	10.106	5.848	5.383	12.230
Swedish 100 ug/ml 2	2916	2994	3.661	4.888	5.048	4.469
Swedish 100 ug/ml 2	3071	2994	3.661	5.207	5.048	4.469
Swedish 100 ug/ml 3	2436	2511	4.197	3.907	4.059	5.288
Swedish 100 ug/ml 3	2585	2511	4.197	4.210	4.059	5.288
Swedish 100 ug/ml 4	5491	5875	9.232	10.287	11.108	10.445
Swedish 100 ug/ml 4	6258	5875	9.232	11.928	11.108	10.445

Table B-6: Calculated concentrations (pg/ml) of IL-8 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-8 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	589277	649353	13.084	3141.947	3556.055	16.469
Amyris 100 ug/ml 1	709428	649353	13.084	3970.162	3556.055	16.469
Amyris 100 ug/ml 2	599473	585279	3.430	3209.453	3116.089	4.237
Amyris 100 ug/ml 2	571084	585279	3.430	3022.724	3116.089	4.237
Amyris 100 ug/ml 3	634925	726156	17.768	3448.092	4113.838	22.886
Amyris 100 ug/ml 3	817387	726156	17.768	4779.585	4113.838	22.886
JP8 10 ug/ml 1	542299	521574	5.620	2837.255	2707.041	6.803
JP8 10 ug/ml 1	500848	521574	5.620	2576.826	2707.041	6.803
JP8 10 ug/ml 2	667472	718323	10.011	3672.662	4041.346	12.902
JP8 10 ug/ml 2	769173	718323	10.011	4410.029	4041.346	12.902
JP8 10 ug/ml 3	451358	450889	0.147	2275.856	2273.055	0.174
JP8 10 ug/ml 3	450420	450889	0.147	2270.254	2273.055	0.174
JP8 100 ug/ml 1	641545	587096	13.116	3493.338	3134.626	16.184
JP8 100 ug/ml 1	532646	587096	13.116	2775.913	3134.626	16.184
JP8 100 ug/ml 2	382460	380199	0.841	1874.227	1861.390	0.975
JP8 100 ug/ml 2	377938	380199	0.841	1848.554	1861.390	0.975
JP8 100 ug/ml 3	497517	495845	0.477	2556.233	2545.917	0.573
JP8 100 ug/ml 3	494172	495845	0.477	2535.602	2545.917	0.573
JP8 100 ug/ml 4	452530	479147	7.856	2282.860	2445.085	9.383
JP8 100 ug/ml 4	505764	479147	7.856	2607.309	2445.085	9.383
JP8 C1	889350	802355	15.334	5356.887	4684.929	20.284
JP8 C1	715360	802355	15.334	4012.971	4684.929	20.284
JP8 C2	512302	458672	16.536	2648.017	2325.851	19.589
JP8 C2	405042	458672	16.536	2003.685	2325.851	19.589
JP8 C4	415243	424826	3.190	2062.857	2119.030	3.749
JP8 C4	434408	424826	3.190	2175.202	2119.030	3.749
JP8 DMSO 1	518514	525913	1.990	2686.872	2733.498	2.412
JP8 DMSO 1	533311	525913	1.990	2780.125	2733.498	2.412
JP8 DMSO 3	492725	496421	1.053	2526.692	2549.497	1.265
JP8 DMSO 3	500117	496421	1.053	2572.303	2549.497	1.265
JP8 DMSO 4	631957	618396	3.101	3427.877	3336.515	3.872
JP8 DMSO 4	604835	618396	3.101	3245.153	3336.515	3.872
Media	306	308	0.918	0.209	0.216	4.229
Media	310	308	0.918	0.222	0.216	4.229
R8 100 2	900781	893601	1.136	5451.552	5392.158	1.558
R8 100 2	886421	893601	1.136	5332.765	5392.158	1.558
R8 100 ug/ml 3	531041	551946	5.356	2765.755	2899.995	6.546
R8 100 ug/ml 3	572851	551946	5.356	3034.236	2899.995	6.546
R8 100 ug/ml 4	585127	577816	1.789	3114.613	3066.785	2.206
R8 100 ug/ml 4	570505	577816	1.789	3018.956	3066.785	2.206
R8 C2	507128	518218	3.026	2615.786	2685.293	3.661
R8 C2	529308	518218	3.026	2754.800	2685.293	3.661
R8 C3	426276	468000	12.608	2127.344	2379.640	14.994
R8 C3	509723	468000	12.608	2631.936	2379.640	14.994
R8 C4	618834	593436	6.053	3339.017	3170.963	7.495
R8 C4	568037	593436	6.053	3002.909	3170.963	7.495
R8 DMSO 2	497788	481849	4.678	2557.906	2460.574	5.594
R8 DMSO 2	465909	481849	4.678	2363.243	2460.574	5.594
R8 DMSO 3	471306	478394	2.095	2395.887	2439.061	2.503
R8 DMSO 3	485482	478394	2.095	2482.235	2439.061	2.503
R8 DMSO 4	384941	388909	1.443	1888.347	1911.013	1.677
R8 DMSO 4	392876	388909	1.443	1933.679	1911.013	1.677

Table B-6 con't: Calculated concentrations (pg/ml) of IL-8 measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: IL-8 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	710056	715046	0.987	3974.685	4010.763	1.272
S8 100 ug/ml 1	720035	715046	0.987	4046.840	4010.763	1.272
S8 100 ug/ml 2	480682	473671	2.093	2452.899	2410.338	2.497
S8 100 ug/ml 2	466660	473671	2.093	2367.778	2410.338	2.497
S8 100 ug/ml 3	433216	470398	11.178	2168.170	2393.380	13.307
S8 100 ug/ml 3	507579	470398	11.178	2618.591	2393.380	13.307
S8 100 ug/ml 4	575278	573486	0.442	3050.070	3038.383	0.544
S8 100 ug/ml 4	571694	573486	0.442	3026.697	3038.383	0.544
S8 C1	754041	760613	1.222	4296.769	4345.919	1.599
S8 C1	767184	760613	1.222	4395.069	4345.919	1.599
S8 C2	474073	509291	9.779	2412.672	2632.012	11.785
S8 C2	544508	509291	9.779	2851.352	2632.012	11.785
S8 C4	805623	776289	5.344	4688.175	4466.168	7.030
S8 C4	746954	776289	5.344	4244.161	4466.168	7.030
S8 DMSO 1	481716	464377	5.280	2459.210	2354.648	6.280
S8 DMSO 1	447038	464377	5.280	2250.086	2354.648	6.280
S8 DMSO 2	717083	705644	2.293	4025.440	3943.299	2.946
S8 DMSO 2	694205	705644	2.293	3861.158	3943.299	2.946
S8 DMSO 3	408888	411480	0.891	2025.944	2040.990	1.043
S8 DMSO 3	414071	411480	0.891	2056.037	2040.990	1.043
Swedish 100 ug/ml 1	596777	618073	4.873	3191.555	3334.998	6.083
Swedish 100 ug/ml 1	639369	618073	4.873	3478.442	3334.998	6.083
Swedish 100 ug/ml 2	506950	572330	16.155	2614.680	3040.886	19.821
Swedish 100 ug/ml 2	637709	572330	16.155	3467.093	3040.886	19.821
Swedish 100 ug/ml 3	498914	495136	1.079	2564.863	2541.571	1.296
Swedish 100 ug/ml 3	491357	495136	1.079	2518.278	2541.571	1.296
Swedish 100 ug/ml 4	761456	796285	6.186	4352.109	4619.690	8.191
Swedish 100 ug/ml 4	831113	796285	6.186	4887.272	4619.690	8.191

Table B-7: Calculated concentrations (pg/ml) of TNF-α measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: TNF-α (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
Amyris 100 ug/ml 1	670	595	17.960	12.669	10.944	22.299
Amyris 100 ug/ml 1	519	595	17.960	9.218	10.944	22.299
Amyris 100 ug/ml 2	877	776	18.510	17.516	15.139	22.206
Amyris 100 ug/ml 2	674	776	18.510	12.762	15.139	22.206
Amyris 100 ug/ml 3	668	695	5.396	12.623	13.237	6.566
Amyris 100 ug/ml 3	721	695	5.396	13.852	13.237	6.566
JP8 10 ug/ml 1	751	663	18.771	14.552	12.520	22.949
JP8 10 ug/ml 1	575	663	18.771	10.488	12.520	22.949
JP8 10 ug/ml 2	924	914	1.626	18.632	18.382	1.920
JP8 10 ug/ml 2	903	914	1.626	18.132	18.382	1.920
JP8 10 ug/ml 3	493	485	2.481	8.633	8.442	3.193
JP8 10 ug/ml 3	476	485	2.481	8.251	8.442	3.193
JP8 100 ug/ml 1	874	809	11.363	17.445	15.916	13.579
JP8 100 ug/ml 1	744	809	11.363	14.388	15.916	13.579
JP8 100 ug/ml 2	612	527	22.966	11.334	9.402	29.052
JP8 100 ug/ml 2	441	527	22.966	7.471	9.402	29.052
JP8 100 ug/ml 3	680	614	15.329	12.900	11.376	18.951
JP8 100 ug/ml 3	547	614	15.329	9.852	11.376	18.951
JP8 100 ug/ml 4	519	508	3.205	9.218	8.959	4.088
JP8 100 ug/ml 4	496	508	3.205	8.700	8.959	4.088
JP8 C1	875	851	4.074	17.468	16.890	4.846
JP8 C1	826	851	4.074	16.311	16.890	4.846
JP8 C2	556	582	6.202	10.056	10.638	7.734
JP8 C2	607	582	6.202	11.219	10.638	7.734
JP8 C4	689	669	4.228	13.109	12.647	5.169
JP8 C4	649	669	4.228	12.184	12.647	5.169
JP8 DMSO 1	892	788	18.766	17.871	15.421	22.475
JP8 DMSO 1	683	788	18.766	12.970	15.421	22.475
JP8 DMSO 3	512	554	10.603	9.060	10.002	13.323
JP8 DMSO 3	595	554	10.603	10.945	10.002	13.323
JP8 DMSO 4	770	701	14.031	14.996	13.383	17.044
JP8 DMSO 4	631	701	14.031	11.770	13.383	17.044
Media	286	267	10.348	4.097	3.686	15.761
Media	247	267	10.348	3.275	3.686	15.761
R8 100 2	1236	977	37.582	26.156	19.958	43.923
R8 100 2	717	977	37.582	13.759	19.958	43.923
R8 100 ug/ml 3	497	633	30.384	8.722	11.847	37.303
R8 100 ug/ml 3	769	633	30.384	14.972	11.847	37.303
R8 100 ug/ml 4	574	669	19.992	10.465	12.649	24.411
R8 100 ug/ml 4	763	669	19.992	14.832	12.649	24.411
R8 C2	554	621	15.258	10.010	11.548	18.831
R8 C2	688	621	15.258	13.086	11.548	18.831
R8 C3	436	444	2.548	7.360	7.538	3.337
R8 C3	452	444	2.548	7.716	7.538	3.337
R8 C4	719	587	31.802	13.806	10.794	39.458
R8 C4	455	587	31.802	7.782	10.794	39.458
R8 DMSO 2	488	474	4.177	8.520	8.207	5.398
R8 DMSO 2	460	474	4.177	7.894	8.207	5.398
R8 DMSO 3	601	581	4.994	11.082	10.614	6.230
R8 DMSO 3	560	581	4.994	10.147	10.614	6.230
R8 DMSO 4	467	449	5.669	8.050	7.650	7.406
R8 DMSO 4	431	449	5.669	7.249	7.650	7.406

Table B-7 con't: Calculated concentrations (pg/ml) of TNF-α measured in the media following 4 h direct exposure to JP-8, Amyris, R-8, S-8 and Swedish Biofuel by individual sample.

4 h direct 7/12: TNF-α (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
S8 100 ug/ml 1	915	783	23.947	18.418	15.312	28.677
S8 100 ug/ml 1	650	783	23.947	12.207	15.312	28.677
S8 100 ug/ml 2	533	528	1.339	9.534	9.421	1.697
S8 100 ug/ml 2	523	528	1.339	9.308	9.421	1.697
S8 100 ug/ml 3	674	576	24.061	12.762	10.529	29.989
S8 100 ug/ml 3	478	576	24.061	8.296	10.529	29.989
S8 100 ug/ml 4	560	575	3.569	10.147	10.477	4.460
S8 100 ug/ml 4	589	575	3.569	10.808	10.477	4.460
S8 C1	865	745	22.779	17.232	14.432	27.435
S8 C1	625	745	22.779	11.632	14.432	27.435
S8 C2	506	570	15.879	8.925	10.382	19.848
S8 C2	634	570	15.879	11.839	10.382	19.848
S8 C4	642	731	17.218	12.023	14.096	20.800
S8 C4	820	731	17.218	16.170	14.096	20.800
S8 DMSO 1	500	593	22.179	8.790	10.915	27.531
S8 DMSO 1	686	593	22.179	13.039	10.915	27.531
S8 DMSO 2	718	631	19.626	13.782	11.772	24.156
S8 DMSO 2	543	631	19.626	9.761	11.772	24.156
S8 DMSO 3	574	545	7.662	10.465	9.797	9.655
S8 DMSO 3	515	545	7.662	9.128	9.797	9.655
Swedish 100 ug/ml 1	660	715	10.879	12.438	13.717	13.185
Swedish 100 ug/ml 1	770	715	10.879	14.996	13.717	13.185
Swedish 100 ug/ml 2	608	640	6.966	11.242	11.967	8.568
Swedish 100 ug/ml 2	671	640	6.966	12.692	11.967	8.568
Swedish 100 ug/ml 3	597	625	6.228	10.990	11.622	7.685
Swedish 100 ug/ml 3	652	625	6.228	12.254	11.622	7.685
Swedish 100 ug/ml 4	1097	905	30.003	22.780	18.223	35.366
Swedish 100 ug/ml 4	713	905	30.003	13.666	18.223	35.366

Table B-8: Calculated concentrations (pg/ml) of GM-CSF measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: GM-CSF (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	4961	4988	0.751	53.455	53.767	0.820
C1	5014	4988	0.751	54.078	53.767	0.820
C4	5391	5452	1.569	58.528	59.245	1.711
C4	5512	5452	1.569	59.961	59.245	1.711
C6	6452	6057	9.223	71.180	66.461	10.042
C6	5662	6057	9.223	61.742	66.461	10.042
JP8 3	8499	7991	9.000	96.048	89.836	9.779
JP8 3	7482	7991	9.000	83.625	89.836	9.779
JP8 4	7798	7040	15.227	87.471	78.306	16.552
JP8 4	6282	7040	15.227	69.141	78.306	16.552
JP8 5	7394	8165	13.354	82.556	91.990	14.504
JP8 5	8936	8165	13.354	101.424	91.990	14.504
Media 2	271	263	4.579	1.860	1.782	6.169
Media 2	254	263	4.579	1.705	1.782	6.169
R8 1	6864	7163	5.903	76.140	79.761	6.420
R8 1	7462	7163	5.903	83.382	79.761	6.420
R8 3	7940	8208	4.618	89.204	92.485	5.017
R8 3	8476	8208	4.618	95.766	92.485	5.017
R8 5	6754	7014	5.242	74.813	77.957	5.702
R8 5	7274	7014	5.242	81.100	77.957	5.702
R8A 1	8618	9492	13.022	97.510	108.335	14.131
R8A 1	10366	9492	13.022	119.159	108.335	14.131
R8A 4	7238	6943	6.009	80.663	77.100	6.536
R8A 4	6648	6943	6.009	73.537	77.100	6.536
R8A 5	6398	5873	12.642	70.532	64.276	13.766
R8A 5	5348	5873	12.642	58.019	64.276	13.766
S8 1	6280	7103	16.386	69.117	79.075	17.809
S8 1	7926	7103	16.386	89.033	79.075	17.809
S8 2	9178	9139	0.604	104.411	103.929	0.655
S8 2	9100	9139	0.604	103.447	103.929	0.655
S8 3	6199	6249	1.132	68.147	68.746	1.232
S8 3	6299	6249	1.132	69.345	68.746	1.232
Swedish 1	11203	12071	10.169	129.633	140.593	11.024
Swedish 1	12939	12071	10.169	151.552	140.593	11.024
Swedish 5	15959	15268	6.400	190.234	181.343	6.934
Swedish 5	14577	15268	6.400	172.453	181.343	6.934
Swedish 8	11591	10614	13.025	134.510	122.295	14.126
Swedish 8	9636	10614	13.025	110.079	122.295	14.126

Table B-9: Calculated concentrations (pg/ml) of IL-1β measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: IL-1β (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	19668	19395	1.991	115.509	113.841	2.071
C1	19122	19395	1.991	112.174	113.841	2.071
C4	23168	23129	0.242	136.960	136.717	0.251
C4	23089	23129	0.242	136.474	136.717	0.251
C6	23682	23794	0.666	140.121	140.810	0.692
C6	23906	23794	0.666	141.499	140.810	0.692
JP8 3	21430	21139	1.947	126.291	124.509	2.025
JP8 3	20848	21139	1.947	122.726	124.509	2.025
JP8 4	21100	21026	0.498	124.269	123.816	0.518
JP8 4	20952	21026	0.498	123.363	123.816	0.518
JP8 5	20875	20084	5.570	122.891	118.055	5.794
JP8 5	19293	20084	5.570	113.218	118.055	5.794
Media 2	284	297	5.962	0.327	0.394	23.983
Media 2	309	297	5.962	0.461	0.394	23.983
R8 1	20284	20022	1.854	119.274	117.670	1.929
R8 1	19759	20022	1.854	116.065	117.670	1.929
R8 3	23350	22989	2.224	138.079	135.857	2.313
R8 3	22627	22989	2.224	133.636	135.857	2.313
R8 5	21994	21518	3.132	129.750	126.829	3.257
R8 5	21041	21518	3.132	123.908	126.829	3.257
R8A 1	20422	20469	0.321	120.119	120.403	0.334
R8A 1	20515	20469	0.321	120.688	120.403	0.334
R8A 4	18849	18926	0.572	110.508	110.975	0.595
R8A 4	19002	18926	0.572	111.442	110.975	0.595
R8A 5	19653	18741	6.886	115.417	109.851	7.165
R8A 5	17828	18741	6.886	104.285	109.851	7.165
S8 1	22586	21859	4.707	133.384	128.922	4.895
S8 1	21131	21859	4.707	124.459	128.922	4.895
S8 2	22485	22467	0.116	132.764	132.650	0.121
S8 2	22448	22467	0.116	132.536	132.650	0.121
S8 3	20525	20762	1.614	120.749	122.200	1.679
S8 3	20999	20762	1.614	123.651	122.200	1.679
Swedish 1	22261	21704	3.633	131.388	127.970	3.778
Swedish 1	21146	21704	3.633	124.551	127.970	3.778
Swedish 5	29805	30326	2.430	177.973	181.211	2.527
Swedish 5	30847	30326	2.430	184.449	181.211	2.527
Swedish 8	20694	21230	3.571	121.783	125.067	3.714
Swedish 8	21766	21230	3.571	128.352	125.067	3.714

Table B-10: Calculated concentrations (pg/ml) of IL-2 measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: IL-2 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	4804	2930	90.452	30.755	18.091	99.000
C1	1056	2930	90.452	5.427	18.091	99.000
C4	890	1833	72.736	4.406	10.526	82.219
C4	2775	1833	72.736	16.645	10.526	82.219
C6	907	982	10.801	4.510	4.971	13.124
C6	1057	982	10.801	5.433	4.971	13.124
JP8 3	1003	1008	0.632	5.099	5.127	0.766
JP8 3	1012	1008	0.632	5.155	5.127	0.766
JP8 4	970	972	0.291	4.896	4.908	0.354
JP8 4	974	972	0.291	4.921	4.908	0.354
JP8 5	922	905	2.657	4.602	4.498	3.262
JP8 5	888	905	2.657	4.394	4.498	3.262
Media 2	263	273	4.930	0.773	0.824	8.708
Media 2	282	273	4.930	0.874	0.824	8.708
R8 1	973	948	3.729	4.914	4.761	4.553
R8 1	923	948	3.729	4.608	4.761	4.553
R8 3	929	962	4.780	4.645	4.844	5.825
R8 3	994	962	4.780	5.044	4.844	5.825
R8 5	927	858	11.462	4.632	4.210	14.173
R8 5	788	858	11.462	3.788	4.210	14.173
R8A 1	822	1009	26.210	3.994	5.146	31.678
R8A 1	1196	1009	26.210	6.299	5.146	31.678
R8A 4	868	880	1.928	4.272	4.346	2.377
R8A 4	892	880	1.928	4.419	4.346	2.377
R8A 5	740	800	10.607	3.500	3.862	13.249
R8A 5	860	800	10.607	4.224	3.862	13.249
S8 1	920	943	3.376	4.589	4.727	4.125
S8 1	965	943	3.376	4.865	4.727	4.125
S8 2	866	909	6.616	4.260	4.520	8.119
S8 2	951	909	6.616	4.779	4.520	8.119
S8 3	1048	1003	6.419	5.377	5.097	7.782
S8 3	957	1003	6.419	4.816	5.097	7.782
Swedish 1	1016	967	7.166	5.179	4.878	8.726
Swedish 1	918	967	7.166	4.577	4.878	8.726
Swedish 5	1024	994	4.342	5.229	5.041	5.270
Swedish 5	963	994	4.342	4.853	5.041	5.270
Swedish 8	911	924	1.914	4.534	4.611	2.345
Swedish 8	936	924	1.914	4.687	4.611	2.345

Table B-11: Calculated concentrations (pg/ml) of IL-5 measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: IL-5 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	908	884	3.922	2.608	2.496	6.387
C1	859	884	3.922	2.383	2.496	6.387
C4	939	952	1.931	2.751	2.811	3.008
C4	965	952	1.931	2.871	2.811	3.008
C6	1145	1158	1.588	3.698	3.758	2.248
C6	1171	1158	1.588	3.818	3.758	2.248
JP8 3	1129	1256	14.300	3.625	4.208	19.608
JP8 3	1383	1256	14.300	4.791	4.208	19.608
JP8 4	1174	1195	2.485	3.831	3.928	3.474
JP8 4	1216	1195	2.485	4.024	3.928	3.474
JP8 5	942	1086	18.752	2.765	3.427	27.316
JP8 5	1230	1086	18.752	4.089	3.427	27.316
Media 2	209	239	17.752	0.000	0.000	NaN
Media 2	269	239	17.752	0.000	0.000	NaN
R8 1	1475	1284	21.100	5.214	4.334	28.704
R8 1	1092	1284	21.100	3.454	4.334	28.704
R8 3	1025	1043	2.374	3.147	3.227	3.526
R8 3	1060	1043	2.374	3.307	3.227	3.526
R8 5	849	888	6.135	2.337	2.514	9.963
R8 5	926	888	6.135	2.691	2.514	9.963
R8A 1	1079	1204	14.630	3.395	3.967	20.394
R8A 1	1328	1204	14.630	4.539	3.967	20.394
R8A 4	995	1093	12.680	3.009	3.459	18.416
R8A 4	1191	1093	12.680	3.909	3.459	18.416
R8A 5	918	978	8.676	2.654	2.930	13.315
R8A 5	1038	978	8.676	3.206	2.930	13.315
S8 1	1106	1077	3.808	3.519	3.386	5.568
S8 1	1048	1077	3.808	3.252	3.386	5.568
S8 2	1065	1106	5.181	3.330	3.517	7.486
S8 2	1146	1106	5.181	3.703	3.517	7.486
S8 3	1161	1073	11.670	3.772	3.365	17.098
S8 3	984	1073	11.670	2.958	3.365	17.098
Swedish 1	1135	1149	1.723	3.652	3.716	2.448
Swedish 1	1163	1149	1.723	3.781	3.716	2.448
Swedish 5	1304	1344	4.158	4.429	4.610	5.565
Swedish 5	1383	1344	4.158	4.791	4.610	5.565
Swedish 8	1240	1162	9.558	4.135	3.774	13.518
Swedish 8	1083	1162	9.558	3.413	3.774	13.518

Table B-12: Calculated concentrations (pg/ml) of IL-6 measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: IL-6 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	122513	116545	7.242	629.194	593.652	8.467
C1	110576	116545	7.242	558.110	593.652	8.467
C4	131793	133596	1.908	685.625	696.723	2.253
C4	135398	133596	1.908	707.821	696.723	2.253
C6	107880	117512	11.592	542.292	599.760	13.551
C6	127144	117512	11.592	657.228	599.760	13.551
JP8 3	281163	278092	1.562	1734.694	1710.416	2.007
JP8 3	275020	278092	1.562	1686.138	1710.416	2.007
JP8 4	247697	232846	9.020	1476.020	1367.096	11.268
JP8 4	217995	232846	9.020	1258.171	1367.096	11.268
JP8 5	205061	208109	2.071	1166.660	1188.096	2.552
JP8 5	211156	208109	2.071	1209.533	1188.096	2.552
Media 2	333	307	12.227	0.452	0.379	27.201
Media 2	280	307	12.227	0.306	0.379	27.201
R8 1	145300	151494	5.782	769.571	809.003	6.893
R8 1	157687	151494	5.782	848.434	809.003	6.893
R8 3	162892	173451	8.609	882.108	952.048	10.389
R8 3	184010	173451	8.609	1021.988	952.048	10.389
R8 5	146776	144309	2.418	778.874	763.375	2.871
R8 5	141842	144309	2.418	747.876	763.375	2.871
R8A 1	242337	231158	6.840	1435.904	1354.142	8.539
R8A 1	219978	231158	6.840	1272.380	1354.142	8.539
R8A 4	157628	167456	8.300	848.054	912.458	9.982
R8A 4	177283	167456	8.300	976.862	912.458	9.982
R8A 5	176059	164933	9.540	968.708	896.120	11.455
R8A 5	153806	164933	9.540	823.532	896.120	11.455
S8 1	173291	177713	3.519	950.334	979.842	4.259
S8 1	182134	177713	3.519	1009.350	979.842	4.259
S8 2	216295	204195	8.380	1246.028	1161.479	10.295
S8 2	192095	204195	8.380	1076.930	1161.479	10.295
S8 3	173767	175629	1.499	953.487	965.865	1.812
S8 3	177490	175629	1.499	978.242	965.865	1.812
Swedish 1	255496	252884	1.461	1535.032	1515.226	1.849
Swedish 1	250272	252884	1.461	1495.420	1515.226	1.849
Swedish 5	328041	330457	1.034	2121.745	2142.547	1.373
Swedish 5	332873	330457	1.034	2163.349	2142.547	1.373
Swedish 8	233089	232496	0.361	1367.525	1363.175	0.451
Swedish 8	231902	232496	0.361	1358.824	1363.175	0.451

Table B-13: Calculated concentrations (pg/ml) of IL-8 measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: IL-8 (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	468639	462527	1.869	3268.210	3222.026	2.027
C1	456415	462527	1.869	3175.843	3222.026	2.027
C4	390629	396965	2.257	2684.166	2731.162	2.433
C4	403301	396965	2.257	2778.158	2731.162	2.433
C6	509427	489148	5.863	3578.667	3424.309	6.375
C6	468869	489148	5.863	3269.951	3424.309	6.375
JP8 3	397854	375332	8.486	2737.714	2571.716	9.128
JP8 3	352810	375332	8.486	2405.717	2571.716	9.128
JP8 4	402455	397584	1.733	2771.872	2735.736	1.868
JP8 4	392713	397584	1.733	2699.600	2735.736	1.868
JP8 5	369819	368517	0.500	2530.563	2520.982	0.537
JP8 5	367214	368517	0.500	2511.401	2520.982	0.537
Media 2	2059	2021	2.659	9.976	9.763	3.076
Media 2	1983	2021	2.659	9.551	9.763	3.076
R8 1	407102	419723	4.252	2806.418	2900.642	4.594
R8 1	432343	419723	4.252	2994.866	2900.642	4.594
R8 3	376466	392100	5.639	2579.524	2695.322	6.076
R8 3	407734	392100	5.639	2811.120	2695.322	6.076
R8 5	533901	454880	24.568	3766.604	3170.823	26.572
R8 5	375858	454880	24.568	2575.041	3170.823	26.572
R8A 1	350896	321077	13.134	2391.709	2175.513	14.054
R8A 1	291258	321077	13.134	1959.318	2175.513	14.054
R8A 4	369569	354156	6.155	2528.724	2415.832	6.609
R8A 4	338742	354156	6.155	2302.940	2415.832	6.609
R8A 5	394488	360151	13.483	2712.753	2460.816	14.479
R8A 5	325814	360151	13.483	2208.880	2460.816	14.479
S8 1	369645	365352	1.662	2529.283	2497.734	1.786
S8 1	361059	365352	1.662	2466.186	2497.734	1.786
S8 2	358700	363888	2.016	2448.878	2486.987	2.167
S8 2	369076	363888	2.016	2525.096	2486.987	2.167
S8 3	504157	483670	5.990	3538.360	3382.652	6.510
S8 3	463183	483670	5.990	3226.944	3382.652	6.510
Swedish 1	399556	375738	8.965	2750.344	2574.773	9.643
Swedish 1	351920	375738	8.965	2399.202	2574.773	9.643
Swedish 5	258503	248073	5.946	1725.322	1651.480	6.323
Swedish 5	237642	248073	5.946	1577.637	1651.480	6.323
Swedish 8	326695	361610	13.655	2215.278	2471.567	14.665
Swedish 8	396525	361610	13.655	2727.855	2471.567	14.665

Table B-14: Calculated concentrations (pg/ml) of TNF-α measured in the media following 18 h vapor exposure to JP-8, R-8, R-8 from algae, S-8 and Swedish Biofuel by individual sample.

18 h vapor 7/20: TNF-α (Human)						
Sample	Signal	Mean	CV	Calc. Concentration	Calc. Conc. Mean	Calc. Conc. CV
C1	1461	1456	0.534	66.233	65.964	0.577
C1	1450	1456	0.534	65.695	65.964	0.577
C4	1602	1476	12.125	73.154	66.960	13.081
C4	1349	1476	12.125	60.767	66.960	13.081
C6	2212	1901	23.136	103.513	88.038	24.858
C6	1590	1901	23.136	72.563	88.038	24.858
JP8 3	1956	1980	1.679	90.696	91.868	1.805
JP8 3	2003	1980	1.679	93.041	91.868	1.805
JP8 4	1944	2291	21.420	90.097	107.573	22.974
JP8 4	2638	2291	21.420	125.049	107.573	22.974
JP8 5	2224	2015	14.707	104.116	93.651	15.803
JP8 5	1805	2015	14.707	83.186	93.651	15.803
Media 2	268	261	4.072	10.148	9.821	4.713
Media 2	253	261	4.072	9.493	9.821	4.713
R8 1	1869	1835	2.620	86.364	84.676	2.820
R8 1	1801	1835	2.620	82.987	84.676	2.820
R8 3	1983	2040	3.918	92.043	94.867	4.211
R8 3	2096	2040	3.918	97.692	94.867	4.211
R8 5	1558	1662	8.810	70.990	76.096	9.490
R8 5	1765	1662	8.810	81.203	76.096	9.490
R8A 1	2262	2154	7.125	106.027	100.584	7.654
R8A 1	2045	2154	7.125	95.140	100.584	7.654
R8A 4	1745	1703	3.488	80.212	78.137	3.757
R8A 4	1661	1703	3.488	76.061	78.137	3.757
R8A 5	1911	1648	22.569	88.454	75.487	24.292
R8A 5	1385	1648	22.569	62.521	75.487	24.292
S8 1	1902	1694	17.411	88.006	77.707	18.743
S8 1	1485	1694	17.411	67.408	77.707	18.743
S8 2	2163	2056	7.360	101.052	95.699	7.910
S8 2	1949	2056	7.360	90.347	95.699	7.910
S8 3	1717	1798	6.333	78.827	82.819	6.817
S8 3	1878	1798	6.333	86.811	82.819	6.817
Swedish 1	2246	2334	5.332	105.222	109.660	5.724
Swedish 1	2422	2334	5.332	114.098	109.660	5.724
Swedish 5	2320	2261	3.690	108.949	105.979	3.963
Swedish 5	2202	2261	3.690	103.010	105.979	3.963
Swedish 8	2191	2458	15.336	102.457	115.941	16.447
Swedish 8	2724	2458	15.336	129.425	115.941	16.447